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*Editor in Chief*
KENNETH T. MORSE

# SOME PROTOZOAN DISEASES OF MAN AND ANIMALS: ANAPLASMOSIS, BABESIOSIS, AND TOXOPLASMOSIS*

*Conference Organizing Committee*
CLARENCE R. COLE, *Chairman*
HILARY KOPROWSKI
ROSS F. NIGRELLI

*Consulting Editor*
ROSS F. NIGRELLI

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* This series of papers is the result of a Conference on *Some Protozoan Diseases of Man and Animals: Anaplasmosis, Babesiosis, and Toxoplasmosis* held by the Section of Biology of The New York Academy of Sciences on November 17 and 18, 1955.

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## MANIFESTATIONS AND DIAGNOSIS OF ANAPLASMOSIS

*By* Hubert Schmidt
*A & M College of Texas College Station Texas*

Anaplasmosis is an infectious disease of cattle caused by *Anaplasma marginale* which infects and destroys the red blood cells The infection frequently terminates fatally but when recovery takes place the blood of the animal permanently retains the infection a condition known as the carrier state Such a carrier is immune for life A small quantity of blood from such a carrier injected into a susceptible bovine will reproduce the disease The destruction of the red blood cells leading to a marked anemia is the outstanding feature of the disease Accordingly when the anemia has progressed far enough we find weakness of the animal which may progress to the point where the animal remains recumbent much of the time and may even be unable to arise developing muscular trembling pallor of the mucous membranes a watery condition of the blood and accelerated respiration and pulse The excessive amount of bile pigment produced by the liver from the destroyed red cells spills over into the blood stream and thus may produce an icteric discoloration of the skin and mucosae Except in rare cases the infection is accompanied by a rise in body temperature Late in the course of the disease one finds anorexia often depression and some salivation especially on hot days Constipation may or may not develop An examination of a stained blood smear will reveal round or roundish purplish stained bodies 0 3 to 0 8 microns in diameter one to three in number located within some of the red cells near the margins

### *The Behavior of the Body Temperature*

There are numerous degrees of intensity of these manifestations varying most markedly with the age of the animal The bovine is susceptible to this disease from birth but an infection of the very young animal does not result in any clinical manifestation whatever and recovery takes place without exception In older animals let us say at the age of 8 months the clinical symptoms will become more apparent so that at this age the animal may show a light fever but nothing else When the animal has reached 12 to 15 months of age or more not only does the fever run very much higher but other symptoms also appear especially anorexia The rate of mortality increases with the advancing age of the animal

This varying rate of mortality reflects varying degrees of severity of clinical manifestations including varying degrees of infection and destruction of red blood cells which can be studied most advantageously in experimentally inoculated animals The amount of blood required for a successful inoculation is so small that transmission of the organism is known to have occurred in herds in which unknown carriers were present during the process of vaccination of the animals for other diseases or during the process of the withdrawal of blood

CONTENTS—(*continued*)

107 F whereas in the mature Jersey the body temperature rarely exceeds 105 F and very frequently does not exceed 104 F even in fatal cases

*Parasitization of the Red Cells*

Red cells parasitized by A *marginale* appear in the blood stream several days before a rise in body temperature occurs At first only a few parasites are found These gradually increase in number so that by the time of the first day of fever from 3 to 48 per cent an average of 16 per cent of the red cells are infected In approximately one half of the cases the number of parasitized red cells increases after the initial day of fever while in the other one half this percentage remains stationary In case of recovery of the animal the number of parasitized red cells gradually recedes and they finally disappear usually within a few days after the body temperature has dropped to normal

Each parasitized cell means a dead cell but how long such a cell will continue to circulate in the blood stream is not known It could be estimated fairly accurately however from a count of the total red cells or the hematocrit were it not for the fact that continuous production of new red cells takes place The *hematocrit drops sharply beginning at the time the first parasitized cells appear in the blood stream and* often it drops faster than the number of parasitized cells increases In some animals it continues to drop even as the percentage of parasitized cells decreases In 17 out of 21 fatal cases the author has found the lowest hematocrit reading to vary between 5 5 and 8 5 the highest reading reaching 13 5 in one animal Recovery may take place with a hematocrit reading as low as 8 The hematocrit readings noted show that from 75 to 80 per cent of the red cells had been destroyed by the parasite

*The Period of Anorexia and Crisis*

In practical animal husbandry it is the keen eye of the owner that recognizes that something is amiss with his best cow As he drives her up from the deep clover meadow in the evening to milk he may notice that she is not quite as full as she usually is and that she looks a little gaunt to him and he may wonder why she has been standing off to one side all by herself He begins to think he can notice a little droopiness about her and she seems to breathe a little faster as he urges her along When he places her customary feed before her she refuses it Now he is sure that something is wrong with her If it is anaplasmosis much has happened to the sick animal by this time but she has shown not the least sign of it unless her milk production has been off a little that morning Prior to this time the animal has been fevering but by now may be free of fever An examination of the blood shows it is more or less watery its staining power is reduced and the microscopical examination of a blood smear reveals the anaplasma bodies This is the typical manner in which the veterinarian is usually confronted with a case of anaplasmosis What has happened to the cow prior to this moment?

The animal has been fevering for from 1 to 4 days but by now the chances are 50 to 50 that she is free of fever Her hematocrit value has dropped from 30 to 10 or 12 showing that more than one half of her red blood cells have been

samples for blood tests when the same needle was used without disinfecting it after use on each animal

During the last 42 years the author has successfully reproduced the disease in thousands of young animals undergoing premunization either for Texas fever which was a double infection of *Piroplasma bigeminum* and *A marginale* or for anaplasmosis alone by the injection of 1 ml blood from a carrier animal These animals were between 8 and 15 months of age and the data cited here for young animals are taken from that work A daily temperature record of the inoculated animal will reveal a rise in body temperature between the 17th and 45th day Rarely will it be below or above that number the greatest number showing their first day of fever between the 30th and 33rd day after inoculation[1] The duration of the fever period in these young animals varies from 2 to 18 days or longer with an average of 7 to 10 days During this time the fever mounts step by step to a maximum and then gradually drops back to normal During the whole of the fever period the rise in body temperature is constant with rare exceptions The mortality rate in animals up to 15 months of age at the time of infection is from 2 to 3 per cent In slightly older animals this rate rises sharply so that in two year olds it may reach 20 per cent This rate increases with the advancing age of the animals so that in fully mature animals it reaches 80 per cent or more

For mature animals the author s data on the period of incubation are not so extensive but according to his records they are approximately the same as for younger animals In a series of 29 cases in mature Jersey cows produced by the author with a strain of *Anaplasma* of more than average virulence the period of incubation ran from 15 to 36 days with an average of 26 days In the majority of these animals this period averaged 2 8 days longer than this average or 29 days The greatest difference in the clinical manifestations of the disease between young animals of the beef breed and mature Jersey cows lies in the duration of the fever period In the young beef breeds this period ranges from 2 to 18 days with an average of 7 to 10 days while in the Jersey cows the fever period ranged from 1 to 5 days with an average of 2 38 days for the cows that died from the disease In the Jersey cows a similar relationship existed in the time between the first day of fever and the occurrence of the lowest hematocrit reading This time varied from 1 to 6 days with an average of 3 37 days for the cows that recovered and from 0 to 5 days with an average of 2 7 days for the cows that died There is also a difference in the behavior of the body temperature of the animal after the peak of fever has been passed Thus a subnormal temperature was recorded before death in 11 out of 21 mature Jersey cows whereas a subnormal temperature was found in only 1 out of 11 comparable animals that recovered In a group of 25 animals of beef breed between 12 and 16 months of age that died from anaplasmosis following artificial inoculation the body temperature shortly before death was subnormal in only one case and had returned to normal in 10 cases while 14 died while they were still fevering

The degree of fever in the different aged animals also varies In the yearling animals a body temperature above 105 F is common with the maximum at

# THE STATUS OF THE COMPLEMENT-FIXATION TEST FOR THE DIAGNOSIS OF ANAPLASMOSIS IN 1955

By D. W. Gates and T. O. Roby
*United States Department of Agriculture, Agricultural Research Service, Animal Disease and Parasite Research Branch, Beltsville, Md.*

The control and eradication of anaplasmosis is recognized as one of the major problems confronting the livestock industry. The detection of the carrier state of bovine anaplasmosis has presented a particularly difficult problem since the blood from a high percentage of carrier animals does not contain sufficient marginal bodies on which to base a diagnosis. The development of the complement fixation test for anaplasmosis, however, has made possible the detection of the carrier, as well as other infected animals.

The test was first applied to the diagnosis of anaplasmosis by Ries and Mohler[1] in 1934. These investigators produced a tick antigen and proved that it was capable of fixing complement. They were unable to produce their antigen in sufficient quantity for experimental testing, however. Mott and Gates[2] developed a crude blood antigen, using a technique similar to that employed by Kent, Bukantz and Rein[3] of the Division of Serology, Army Medical School, Army Medical Center, Washington, D. C., in the development of a malaria antigen. This antigen could be produced in quantity but had the disadvantage of containing two much color and many lots were anticomplementary. Later a carbon dioxide precipitate antigen was prepared by the same workers, using a technique developed by Heidelberger and Mayer.[4] This process removed most of the color and resulted in a more uniform antigen. Many of these antigens were also anticomplementary, however.

It soon became apparent that the test was capable of giving accurate results but the main problem was the production of a satisfactory antigen. From this time forward, antigen research was directed toward the use of cattle infected with anaplasmosis for the reason that no practical means of propagating the organism other than in bovine blood have been developed.

Price, Poelma, and Faber[5] of the Maryland Livestock Sanitary Service, College Park, Md., in 1952 produced an antigen in which the red cells were lysed with distilled water. This antigen was more concentrated and less anticomplementary than the carbon dioxide antigen.

Gates and his co-workers[6] reported the results of work done by the Maryland Livestock Sanitary Service and the Animal Disease and Parasite Research Branch of the United States Department of Agriculture. Three types of anaplasmosis antigens, designated as carbon dioxide precipitate antigen ($CO_2$ antigen), Servall distilled water extract antigen (Servall antigen), and Sharples distilled water extract antigen (Sharples antigen) were compared. They were produced from infective material from the same donor animals and were tested by the United States Department of Agriculture and by the Maryland laboratories using the same testing reagents. Each type of antigen was satisfactory for use. The production methods were compared for productivity, economy, and practical application. Productivity comparison, based on the number of

destroyed, and that from 10 to 30 per cent of those remaining are parasitized by *A marginale* The chances of her recovery are not very good The crisis is already at hand before the animal gives evidence of sickness the first definite sign of which is usually anorexia Mature animals survive from 1 to 4 days average 2½ days after its onset during which time the body temperature has returned to normal if it had not already done so on the first day of the period or it even becomes subnormal Thus 9 out of 21 mature Jersey cows survived 1 to 5½ days after the temperature had returned to normal and all 9 developed anorexia after the fever had subsided and the animals died The animal remains recumbent much of the time and shows other signs of severe anemia as already detailed The surprising features are the shortness of the survival period after anorexia becomes manifest and the normal or subnormal temperature In immature animals the temperature rarely drops to subnormal before death in less than one half of the fatal cases it has returned to normal while the majority die while still fevering In these animals contrary to the course of the disease in mature animals the period of anorexia is sometimes very long It is not unusual for the anorexia to last a week while the maximum period observed by the writer was two weeks followed by recovery Sometimes these young animals get so weak that they remain recumbent unable to rise for several days and yet they recover

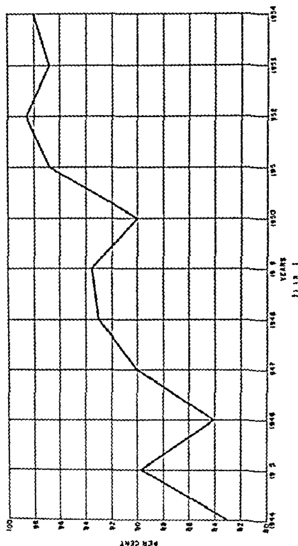
## *Diagnosis*

Since the first symptom of anaplasmosis in an animal recognized by its owner is a loss of appetite and since in a large percentage of cases the period of fever has already passed and the body temperature has returned to normal before anorexia develops it is important that one be on guard not to take this anorexia as a symptom of some afebrile condition A microscopic examination of the blood will readily eliminate such an error The *Anaplasma* bodies can readily be found if properly stained and a hematocrit reading will verify the fact that a destruction of red blood cells has occurred It is mandatory that the *Anaplasma* bodies be demonstrated Since icterus does not always develop it can arouse suspicion only in case of its presence

At autopsy the outstanding feature is the greatly enlarged spleen with its soft to reddish brown pulpa and the enlarged gall bladder filled with dark grumous bile The liver is enlarged with rounded edges and if icterus had developed of a yellowish color Further one finds degeneration of the heart muscle frequently epicardial petechiation and a moderate gastrointestinal catarrh

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ACCURACY OF THE COMPLEMENT-FIXATION TEST
FOR
THE DIAGNOSIS OF BOVINE ANAPLASMOSIS
PER CENT
YEARS
100
80
1944
1946
1948
1950
1954

test units obtained from one cc of blood were Servall 1 367 test units Sharples 1 223 test units and CO method 0 900 test units Economy of production was based on the man hours of work required for processing one liter of blood and were Sharples 2 4 man hours CO 3 0 man hours and Servall 4 3 man hours The practical application or summary comparison was made on the basis of productivity economy adaptability for large scale production anti complementary activity, test accuracy and all other factors known about each method On the above basis the production methods were ranked Sharples first $CO_2$ second and Servall third

As a result of these studies antigen production has been put on a firm basis The carbon dioxide and Sharples antigen production methods have been employed to produce in excess of 1 5 million test doses This antigen is being used at the present time in field work to determine the incidence of the disease Also antigen is being used in an experimental eradication program in the Hawaiian Islands

The complement fixation method as used for the diagnosis of bovine anaplasmosis is similar to that employed in our laboratory for many years in the large scale testing of equine sera for dourine and glanders Essentially, this procedure is that described by Mohler and Eichhorn[7] for the diagnosis of glanders Modifications of the method as applied to anaplasmosis will be published in the proceedings of the 59th annual meeting (1955) United States Livestock Sanitary Association College Park Md

*Accuracy of the Test*

The accuracy of the complement fixation test for bovine anaplasmosis has long been the subject of investigation In 1949 a high degree of accuracy was shown by the work of Gates Mohler Poelma and Hastings[8] who made a number of inoculations into splenectomized calves Blood samples from 119 negative animals were injected into 10 splenectomized calves all of which remained normal A splenectomized calf was inoculated from each of nine positive animals selected at random Seven of these calves developed acute anaplasmosis and two remained normal Two splenectomized calves were inoculated with blood from animals giving a suspicious reaction One calf developed acute anaplasmosis and one remained normal

More than 6 000 tests have been applied to sera collected from normal acute and carrier animals located in the anaplasmosis experimental herd at the Animal Disease Station Beltsville Md An accurate record has been kept on this herd for the past 28 years Splenectomies and inoculations were made when necessary in order to determine the status of each animal It was found that serum preserved with phenol gave the most satisfactory results A final concentration of 0 5 per cent phenol is made by adding one part of 5 per cent aqueous phenol solution to 9 parts of serum The yearly per cent accuracy has gradually increased during the past 11 years Methods for calculating accuracy determinations are described in a report by Gates and his co workers[8] The average per cent for the 11 year period was 92 3 per cent as shown in FIGURE 1 This compares favorably with other acceptable serological tests The results of 1954 testing are shown in TABLE 1

ANAPLASMOSIS

COMPLEMENT FIXATION RESULTS ON SERUM COLLECTED FROM SPLENECTOMIZED CALF # 3763

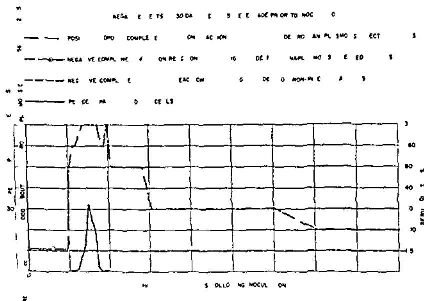


Fig 2

plied the antigen for testing In addition to this testing the states of Mary land Oklahoma and Kansas are producing antigen and are doing some testing The planned program for the development of testing includes the training of state technicians six of which have been trained in our laboratory to date In conducting these surveys it is the policy of the Animal Disease and Parasite Research Branch to do some preliminary testing Following this the state sends a technician to our laboratory where he is trained to perform the comple ment fixation test When the training is finished the technician goes back to his home state and proceeds to set up a testing laboratory The Animal Dis ease and Parasite Research Branch maintains a supply of antigen and ships it to the testing laboratories on request A temporary supply of the other re agents is usually sent to the new laboratories to start testing In addition to these reagents a supply of reference positive and negative sera are furnished the new laboratory Initially the serologist submits his sera for check testing until his results appear consistently valid When the serologist feels he is ready to start testing a number of coded samples are forwarded to him These serum samples have been collected from animals with a known anaplasmosis history They are phenolized and a code number is assigned to each sample at the Ani mal Disease Station These samples are first tested at our laboratory to verify their status and then forwarded to the state laboratory for testing A complete record of the coding system is kept on file at Beltsville and the serologist at the

Table 1

Results of the Anaplasmosis Complement Fixation Test on Serum Samples from Known Positive and Negative Animals Collected in 1954

| | Positive samples | | | | | Negative samples | | | | | Total titles | Average per cent |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4+ | 3+ | 2+ | + | − | − | + | 2+ | 3+ | 4+ | | |
| January | 9 | — | — | — | — | 23 | — | — | — | — | 32 | 100 0 |
| February | 7 | — | — | — | 1 | 28 | — | — | — | — | 36 | 97 22 |
| March | 8 | — | — | — | — | 31 | — | — | — | — | 39 | 100 0 |
| April | 8 | — | — | — | — | 31 | — | — | — | — | 39 | 100 0 |
| May | 6 | — | — | — | 1 | 31 | — | — | — | 1 | 39 | 94 8[illegible] |
| June | 7 | — | — | — | — | 33 | — | — | — | — | 40 | 100 0 |
| July | 6 | — | — | — | — | 31 | 2 | — | — | 1 | 40 | 96 25 |
| August | 5 | 1 | — | — | — | 40 | 1 | — | — | — | 47 | 98 93 |
| September | 6 | — | — | — | — | 38 | — | — | — | 1 | 45 | 97 77 |
| October | 6 | — | — | — | — | 34 | 1 | 1 | — | 1 | 43 | 94 76 |
| November | 5 | — | — | 1 | — | 35 | — | — | — | — | 41 | 98 17 |
| December | 5 | — | 1 | — | — | 42 | 1 | — | — | — | 49 | 98 46 |
| Totals | 78 | 1 | 1 | 1 | 2 | 397 | 5 | 1 | — | 4 | 490 | 98 1 |

A total of 83 serum samples were collected from infected animals 2 of which gave false negative reactions and 3 gave reactions less than a 4+ Of 407 sera collected from normal animals 397 gave negative reactions five gave 1+ one 2+ and four a 4+ reading The yearly average of accuracy was 98 1 per cent

*Specificity*

It has been questioned whether infection with or vaccination against other diseases may result in cross reactions with anaplasmosis Recent studies have been made to assess the specificity of the test on sera collected from animals infected with other common bovine diseases and also on cattle vaccinated for brucellosis These studies are being reported in detail in another paper[9] There were no cross reactions observed when sera from cattle infected with brucellosis vibriosis and vesicular stomatitis were tested Of 204 noninfected animals vaccinated for brucellosis 80 per cent of which had a positive Brucella agglutinin titer 202 were negative 1 suspicious and 1 positive to the anaplasmosis test The cause for reactions in these two animals is as yet undetermined The serum which gave a positive reaction to the anaplasmosis test was negative for brucellosis however and blood from the positive reactor failed to infect an inoculated splenectomized calf This type of reaction is being studied The specific nature of the test has been further studied in connection with the use of antigens made from noninfected animals Figures 2 and 3 indicate reactions obtained when sera from experimentally infected animals were tested with specific and control antigens No reactions with serum from known infected animals have been encountered when these control antigens were tested

*Field Surveys*

Field disease incidence surveys have been conducted in seven states for which the Animal Disease and Parasite Research Branch has done the testing or up

ANAPLASMOSIS

COMPLEMENT-FIXATION RESULTS ON SERUM COLLECTED FROM SPLENECTOMIZED CALF # 3763

plied the antigen for testing In addition to this testing the states of Maryland Oklahoma and Kansas are producing antigen and are doing some testing The planned program for the development of testing includes the training of state technicians six of which have been trained in our laboratory to date In conducting these surveys it is the policy of the Animal Disease and Parasite Research Branch to do some preliminary testing Following this the state sends a technician to our laboratory where he is trained to perform the complement fixation test When the training is finished the technician goes back to his home state and proceeds to set up a testing laboratory The Animal Disease and Parasite Research Branch maintains a supply of antigen and ships it to the testing laboratories on request A temporary supply of the other reagents is usually sent to the new laboratories to start testing In addition to these reagents a supply of reference positive and negative sera are furnished the new laboratory Initially the serologist submits his sera for check testing until his results appear consistently valid When the serologist feels he is ready to start testing a number of coded samples are forwarded to him These serum samples have been collected from animals with a known anaplasmosis history They are phenolized and a code number is assigned to each sample at the Animal Disease Station These samples are first tested at our laboratory to verify their status and then forwarded to the state laboratory for testing A complete record of the coding system is kept on file at Beltsville and the serologist at the

TABLE 1
RESULTS OF THE ANAPLASMOSIS COMPLEMENT FIXATION TEST ON SERUM SAMPLES FROM KNOWN POSITIVE AND NEGATIVE ANIMALS COLLECTED IN 1954

| | Positive samples | | | | | Negative samples | | | | | Total title | Average per cent |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4+ | 3+ | 2+ | + | – | – | + | 2+ | 3+ | 4+ | | |
| January | 9 | — | — | — | — | 23 | — | — | — | — | 32 | 100.0 |
| February | 7 | — | — | — | 1 | 28 | — | — | — | — | 36 | 97.22 |
| March | 8 | — | — | — | — | 31 | — | — | — | — | 39 | 100.0 |
| April | 8 | — | — | — | — | 31 | — | — | — | — | 39 | 100.0 |
| May | 6 | — | — | — | 1 | 31 | — | — | — | 1 | 39 | 94.87 |
| June | 7 | — | — | — | — | 33 | — | — | — | — | 40 | 100.0 |
| July | 6 | — | — | — | — | 31 | 2 | — | — | 1 | 40 | 96.25 |
| August | 5 | 1 | — | — | — | 40 | 1 | — | — | — | 47 | 98.93 |
| September | 6 | — | — | — | — | 38 | — | — | — | 1 | 45 | 97.77 |
| October | 6 | — | — | — | — | 34 | 1 | 1 | — | 1 | 43 | 94.76 |
| November | 5 | — | — | 1 | — | 35 | — | — | — | — | 41 | 98.17 |
| December | 5 | — | 1 | — | — | 42 | 1 | — | — | — | 49 | 98.46 |
| Totals | 78 | 1 | 1 | 1 | 2 | 397 | 5 | 1 | — | 4 | 490 | 98.1 |

A total of 83 serum samples were collected from infected animals, 2 of which gave false negative reactions and 3 gave reactions less than a 4+. Of 407 sera collected from normal animals, 397 gave negative reactions, five gave 1+, one 2+, and four a 4+ reading. The yearly average of accuracy was 98.1 per cent.

*Specificity*

It has been questioned whether infection with or vaccination against other diseases may result in cross reactions with anaplasmosis. Recent studies have been made to assess the specificity of the test on sera collected from animals infected with other common bovine diseases and also on cattle vaccinated for brucellosis. These studies are being reported in detail in another paper.[9] There were no cross reactions observed when sera from cattle infected with brucellosis, vibriosis, and vesicular stomatitis were tested. Of 204 noninfected animals vaccinated for brucellosis, 80 per cent of which had a positive Brucella agglutinin titer, 202 were negative, 1 suspicious, and 1 positive to the anaplasmosis test. The cause for reactions in these two animals is as yet undetermined. The serum which gave a positive reaction to the anaplasmosis test was negative for brucellosis, however, and blood from the positive reactor failed to infect an inoculated splenectomized calf. This type of reaction is being studied. The specific nature of the test has been further studied in connection with the use of antigens made from noninfected animals. FIGURES 2 and 3 indicate reactions obtained when sera from experimentally infected animals were tested with specific and control antigens. No reactions with serum from known infected animals have been encountered when these control antigens were tested.

*Field Surveys*

Field disease incidence surveys have been conducted in seven states for which the Animal Disease and Parasite Research Branch has done the testing for up

nectomized calves The Hawaiian cattle population consists of about 12 000 dairy animals and 150 000 beef cattle Preliminary testing revealed that 3 9 per cent of 7 000 dairy animals and 1 6 per cent of 5 300 beef cattle reacted to the complement fixation test for anaplasmosis As a result of this testing the Hawaiian government decided to institute an eradication program and arrangements were made for the Hawaiian Islands and the United States government to pay indemnity for cattle reacting to the test Up to the present time a total of 40 939 animals have been tested 98 92 per cent or 40 497 were negative 0 30 per cent or 126 suspicious and 0 77 per cent or 316 positive Originally all the testing in the Hawaiian Islands was done in our laboratory One man trained in our laboratory established the test in a laboratory on the islands and is now doing all the testing there Briefly, the following regulations were adopted in September 1954 to prevent the spread of anaplasmosis infection all reactors are to be removed within 15 days after being identified no additions are to be made until after the removal of the reactors all herd additions shall pass two clean tests at not less than 60 days interval except that cattle from herds officially declared free of anaplasmosis shall require one negative test

Insect vectors are not considered to be a major problem in the spread of the disease under Hawaiian conditions It is the opinion of the Hawaiian livestock officials that anaplasmosis can be eradicated from the islands in approximately two years provided sufficient testing is done A summary of the tests conducted on field sera from August 1954 to October 1955 is shown in TABLE 2

### *Future Work*

The test has been developed to the point where it is now accepted as a valuable diagnostic agent for research investigations It remains however to be tested under field experimental conditions to determine its value in control and eradication programs A carefully thought out plan should be provided for this work Any plan of testing should be under the control of the federal and state livestock authorities Experience has proved that the best results have

TABLE 2
ANAPLASMOSIS COMPLEMENT FIXATION TESTS PERFORMED ON FIELD SERA AUGUST 1954 TO OCTOBER 1955

| State or territory | Negative | Suspicious | Positive | Total | Per cent positive |
|---|---|---|---|---|---|
| California (Hawaiian imports) | 758 | 37 | 54 | 849 | 6 36 |
| Missouri | 453 | 9 | 89 | 551 | 16 15 |
| Oregon (eastern) | 1714 | 141 | 1558 | 3413 | 45 64 |
| Tennessee | 214 | 16 | 53 | 283 | 18 72 |
| Virginia | 3104 | 40 | 34 | 3178 | 1 06 |
| Washington (Hawaiian imports) | 264 | | | 264 | |
| Hawaii | | | | | |
| U S laboratory | 14779 | 96 | 294 | 15169 | 1 9 |
| Hawaii laboratory | 25718 | 30 | 22 | 25 70 | 08 |
| | 47004 | 369 | 2104 | 49477 | |

T t b g d t d th t t f M ryl d M t Okl h m d K f wh h w h dat

ANAPLASMOSIS

COMPLEMENT-FIXATION RESULTS ON SERUM COLLECTED FROM SPLENECTOMIZED CALF #3958

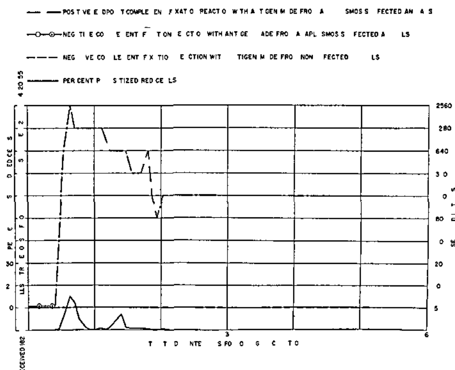



FIGURE 3

new laboratory does not have access to this record When a satisfactory report on the coded samples has been received the laboratory is ready to do field testing and is required to forward all positive and an equal number of negative samples to the Animal Disease Station for check testing The serologist reports his findings at regular intervals to the Animal Disease and Parasite Research Branch at Beltsville where they are tabulated During the past 14 months surveys have been conducted in six states A total of approximately 8 500 cattle were tested Of this number 6 507 were negative 243 suspicious and 1 788 positive The incidence of the disease ranged from no reactors in the state of Washington to 45 6 per cent in eastern Oregon Tests are also being conducted in the states of Maryland Montana Oklahoma and Kansas for which we have incomplete or no data

*Hawaiian Program*

Several cases of suspected anaplasmosis occurred on the Hawaiian Islands in May 1954 The diagnosis was later confirmed by subinoculations into sple

nectomized calves. The Hawaiian cattle population consists of about 12,000 dairy animals and 150,000 beef cattle. Preliminary testing revealed that 3.9 per cent of 7,000 dairy animals and 1.6 per cent of 5,300 beef cattle reacted to the complement fixation test for anaplasmosis. As a result of this testing the Hawaiian government decided to institute an eradication program and arrangements were made for the Hawaiian Islands and the United States government to pay indemnity for cattle reacting to the test. Up to the present time a total of 40,939 animals have been tested: 98.97 per cent or 40,497 were negative, 0.30 per cent or 126 suspicious and 0.77 per cent or 316 positive. Originally all the testing in the Hawaiian Islands was done in our laboratory. One man trained in our laboratory established the test in a laboratory on the islands and is now doing all the testing there. Briefly, the following regulations were adopted in September 1954 to prevent the spread of anaplasmosis infection: all reactors are to be removed within 15 days after being identified; no additions are to be made until after the removal of the reactors; all herd additions shall pass two clean tests at not less than 60 days interval, except that cattle from herds officially declared free of anaplasmosis shall require one negative test.

Insect vectors are not considered to be a major problem in the spread of the disease under Hawaiian conditions. It is the opinion of the Hawaiian livestock officials that anaplasmosis can be eradicated from the islands in approximately two years provided sufficient testing is done. A summary of the tests conducted on field sera from August 1954 to October 1955 is shown in TABLE 2.

*Future Work*

The test has been developed to the point where it is now accepted as a valuable diagnostic agent for research investigations. It remains, however, to be tested under field experimental conditions to determine its value in control and eradication programs. A carefully thought out plan should be provided for this work. Any plan of testing should be under the control of the federal and state livestock authorities. Experience has proved that the best results have

TABLE 2
ANAPLASMOSIS COMPLEMENT FIXATION TESTS PERFORMED ON FIELD SERA AUGUST 1954 TO OCTOBER 1955

| State or territory | Negative | Suspicious | Positive | Total | Per cent positive |
|---|---|---|---|---|---|
| California (Hawaiian imports) | 758 | 37 | 54 | 849 | 6.36 |
| Missouri | 453 | 9 | 89 | 551 | 16.15 |
| Oregon (eastern) | 1714 | 141 | 1558 | 3413 | 45.64 |
| Tennessee | 214 | 16 | 53 | 283 | 18.72 |
| Virginia | 3104 | 40 | 34 | 3178 | 1.06 |
| Washington (Hawaiian imports) | 264 | | | 264 | |
| Hawaii | | | | | |
| U. S. laboratory | 14779 | 96 | 294 | 15169 | 1.9 |
| Hawaii laboratory | 25718 | 30 | 22 | 25770 | .08 |
| | 47004 | 369 | 2104 | 49477 | |

[illegible] Maryland [illegible] Oklahoma [illegible]

been obtained in control programs when the diagnostic reagents, such as antigen have been prepared and standardized in a central laboratory It has not yet been conclusively determined whether anaplasmosis can be eradicated by segregation or slaughter of reacting animals Additional representative areas of the United States should be surveyed in order to obtain a true incidence picture of the disease The following proposed experimental field evaluation studies and accompanying regulations are suggested for federal state cooperative work It is planned to test individual herds totaling 8 000 or more cattle and small areas such as townships in areas where the incidence is low as well as other similar size areas where the incidence is high The value of insecticides in these control studies needs reinvestigation Insecticides should be used on one half of the herds in a highly infected area with no insecticides in similar herds in this particular group Another group of herds should be tested in which the reactors in half the herds are isolated from the negative animals In the balance of the herds in this group the reactors should remain in the herds The quarantine regulations recommended for these studies are as follows (1) retest all clean herds annually (2) retest all infected herds at not less than 60 day intervals (3) retest semiannually all clean herds that contained infection the previous year (4) hold all herd additions in quarantine until they have passed two clean tests at not less than 60 day intervals (5) pass all clean herds through two clean tests over a period of a year before declaring them free of anaplasmosis (6) pass all infected herds through three clean tests over a period of a year before declarin them free of anaplasmosis (7) identify and brand all reactors


*Summary*

The procedure of the complement fixation test for anaplasmosis is essentially the same as that originally used by the United States Bureau of Animal Industry for the diagnosis of dourine and glanders with the exception of the antigen Antigen is being produced in volume for incidence surveys and experimental eradication programs This antigen when used in the complement fixation test for the diagnosis of anaplasmosis is 98 per cent accurate An experimental eradication program is being conducted in the Hawaiian Islands where it is believed the disease can be eradicated in a comparatively short time

Studies made to evaluate the specificity of the te t showed no cross reactions with sera collected from animals affected with other diseases and cattle vaccinated for brucellosis

Limited field surveys have shown that the incidence of the disease varies up to 45 per cent in certain areas of the United States

Future work is planned to determine practical methods for controlling the disease in areas where it is endemic



*Acknowledgment*

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# TRANSMISSION OF ANAPLASMOSIS

*By* Paul L Piercy

*Department of Physiology and Pharmacology University of Georgia Athens Ga*

Compared to some diseases the history of anaplasmosis dates back through a relatively short period of time Nevertheless the occurrence of the malady has produced major challenges to investigators in many parts of the world One of the most interesting of these challenges was also one of the first to attract the curiosity of investigators and one that has continued to receive concentrated attention since the disease was first recognized as a specific entity 45 years ago It is concerned with the means by which the disease is perpetuated and it has precipitated large numbers of experimental transmission studies, the results of which have helped to define the problems of anaplasmosis more distinctly Although various agents have been incriminated as potential vectors in nature, an exhaustive study of all possible vectors has not been made

The agencies befitting the role of natural vectors are arthropods of numerous genera and species including ticks so called horseflies and mosquitoes Certain of these are biological vectors and the remainder demonstrate the qualities of mechanical vectors Man also is responsible for accidental mechanical transmission at times when inadequate precautions are taken to prevent the transfer of blood between animals in the performance of common herd operative procedures In addition it is indicated that the disease is occasionally passed on from dam to fetus *in utero*

The purpose of this discussion shall be to compare the probable relative roles of transmitting agencies The comparison will be based largely upon the epizootiology of the disease as observed especially in the southern and southwestern segments of the United States where the clinical incidence of anaplasmosis is enzootic in nature Although the disease has been reported at times from many states outside of this area its occasional appearance there frequently can be traced back to the acquisition of carrier animals originating from infected areas A carrier animal is defined as one that has recovered from an attack of the disease, whether it be mild or severe and no longer reveals clinical or definite microscopic manifestations Except in very rare instances such animals harbor the etiologic agent of anaplasmosis in their blood streams and in consequence are latent infection reservoirs probably throughout the remainder of their lives The evidence to be presented supports the contention that Tabanidae are probably the most important in the southern United States It is not meant to imply however that ticks and other blood sucking arthropods might not be the most important natural vectors in other geographic areas It seems reasonable to assume that probably most blood sucking insects should be considered as potential vectors that may transmit anaplasmosis within the range of their distribution if conditions for their feeding are optimal and if they are active in sufficient numbers

### *Transmission by Ticks*

Smith and Kilborne[1] reported that *Piroplasma bigeminum* the causative agent of piroplasmosis in cattle was transmitted by *Boophilus annulatus*

in the United States They also observed abnormal microscopic structures other than *P bigeminum* in the blood of some of the animals they studied describing them as 'peripheral coccuslike bodies that were often present in the erythrocytes following the occurrence of piroplasmosis They assumed that these intracellular structures represented a late developmental stage of *P bigeminum* and believed that the accompanying clinical manifestations were indicative of a secondary reaction to piroplasmosis Dikmans[2] later made a careful analysis of the results of the transmission studies reported by Smith and Kilborne and found evidence that they had been successful in transmitting both piroplasmosis and anaplasmosis with *B annulatus* in at least one animal That animal reacted to piroplasmosis beginning 17 days after infected larvae were placed on it and to anaplasmosis 56 days after placement of the ticks The peripheral cocci were observed to be present in 15 to 20 per cent of the erythrocytes on January 16 1891 This date would thus put on record the first known successful experimental tick transmission of anaplasmosis The peripheral coccuslike bodies described were those that are now recognized as *Anaplasma marginale*

Theiler's work[3] in South Africa reported in 1910 definitely identified anaplasmosis as a disease *sui generis* and gave support to the fact that Smith and Kilborne had unknowingly incriminated *B annulatus* as a natural vector of both anaplasmosis and piroplasmosis Theiler[4] later reported the successful transmission of anaplasmosis by *B decoloratus* with incubation periods varying from 52 to 100 days and by *Rhipicephalus simus* with an incubation period of 75 days

*B annulatus* was the only known natural vector of piroplasmosis and anaplasmosis in the United States at the time the latter was identified as a specific disease This suggested a possibility that both diseases might be eliminated by eradicating that one vector The tick eradication program subsequently proved successful in eliminating piroplasmosis but anaplasmosis remained and it was apparent that vectors other than *B annulatus* must also be capable of transmitting that malady This hypothesis stimulated extensive investigation of the natural transmission of anaplasmosis in subsequent years in the hope of constructing an effective vector eradication pattern for eliminating that disease also For many years investigators concentrated on the role played by ticks An excellent review and tabulation of tick transmission studies by Dikmans[5] and the listing of experimentally capable tick vectors by Stiles[6] reveals the eventual incrimination of 7 genera and 19 species of experimental tick vectors of anaplasmosis In alphabetical sequence they are *Argas persicus Boophilus annulatus B calcaratus B decoloratus B microplus Dermacentor albipictus D andersoni D occidentalis D variabilis Hyalomma lusitanicum H aegyptium Ixodes ricinus I scapularis Ornithodorus lahorensis Rhipicephalus appendiculatus R bursa R evertsi R sanguineus and R simus*

Dikmans tabulation reveals that transovarian transfer of the etiologic agent from the engorged female tick through the egg to the larval stage has been accomplished under experimental conditions by *Boophilus annulatus B decoloratus B microplus Dermacentor andersoni D occidentalis Ixodes ricinus* and *Rhipicephalus simus* Mechanical transfer feedings that involved an interrup

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The agencies befitting the role of natural vectors are arthropods of numerous genera and species, including ticks, so called horseflies and mosquitoes. Certain of these are biological vectors, and the remainder demonstrate the qualities of mechanical vectors. Man also is responsible for accidental mechanical transmission at times when inadequate precautions are taken to prevent the transfer of blood between animals in the performance of common herd operative procedures. In addition, it is indicated that the disease is occasionally passed on from dam to fetus *in utero*.

The purpose of this discussion shall be to compare the probable relative roles of transmitting agencies. The comparison will be based largely upon the epizootiology of the disease as observed especially in the southern and southwestern segments of the United States, where the clinical incidence of anaplasmosis is enzootic in nature. Although the disease has been reported at times from many states outside of this area, its occasional appearance there frequently can be traced back to the acquisition of carrier animals originating from infected areas. A carrier animal is defined as one that has recovered from an attack of the disease, whether it be mild or severe, and no longer reveals clinical or definite microscopic manifestations. Except in very rare instances, such animals harbor the etiologic agent of anaplasmosis in their blood streams and, in consequence, are latent infection reservoirs probably throughout the remainder of their lives. The evidence to be presented supports the contention that Tabanidae are probably the most important in the southern United States. It is not meant to imply, however, that ticks and other blood sucking arthropods might not be the most important natural vectors in other geographic areas. It seems reasonable to assume that probably most blood sucking insects should be considered as potential vectors that may transmit anaplasmosis within the range of their distribution if conditions for their feeding are optimal and if they are active in sufficient numbers.

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dergo digestion in the tick and because the causative agent of the disease is still retained in one or more subsequent cyclical forms of the vector the biological survival time in the tick must necessarily be long The means by which this etiologic agent is protected against destruction under these circumstances presents a perplexing problem

Ticks yielding negative transmission results have been reported by Sanborn and Moe[10] to include *Amblyomma americanum A cajennense Dermacentor nitens D parumapertus D venustus Hemaphysalis leporis palustris Ixodes sculptus Ornithodorus megnini* and *O turicata* The negative results that they reported for *A americanum* have been confirmed by Piercy and Schmidt[11,12] and by Rees[13] These workers were also unable to demonstrate transmission with *A maculatum* Both of these *Amblyomma* species have been tested in all stages of development using both carrier and clinically infected animals as the infection reservoirs These ticks have received special attention because they are very prevalent in southern United States and infest cattle freely Although their feeding habits developmental cycles environmental preferences and other qualities are closely comparable to other ticks it has not been possible to incriminate them as vectors of anaplasmosis

*Transmission by Flies*

The persisting enzootic occurrence of anaplasmosis in the southern United States along with the known presence there of potential natural vectors other than ticks brought about a more intensive investigation of transmitting agencies Evidence accumulated during the past 25 years has increasingly incriminated horseflies especially Tabanidae species as an extremely important class of natural vectors of the disease The first report of experimental transmission by flies was made by Sanborn *et al*[14] and dates back to 1930 These workers succeeded in transmitting anaplasmosis to a susceptible cow by a total of 41 composite transfer feedings with *Tabanus gracilis T sulcifrons* and *Chrysops sequax*

Certain enzootic features of anaplasmosis were observed by the author during consecutive seasons of tick and fly transmission study in the Texas gulf coast region The occurrence of the disease followed a typical seasonal pattern in which the greatest clinical incidence was from June to September or October The peak in incidence and the greatest regional distribution were reached during August and early September The incidence in other months of the year was minimal and isolated During years of excessive rainfall the clinical incidence and distribution was markedly greater than during years in which seasonal precipitation approached the average Coincidentally the populations of Tabanidae were closely correlated with the incidence of anaplasmosis Few cases of the disease were observed before the fly season or several weeks after horseflies had disappeared Further there was no obvious relationship between the incidence of anaplasmosis and the prevailing tick and mosquito populations

The natural feeding habits of Tabanidae provide a source of considerable torment to host animals The dermal puncture wounds they make evidently produce some degree of pain and after engorgement is completed and the mouth

tion of engorgement on infected animals and transfer of the ticks to permit completion of engorgement on susceptible animals by *Boophilus calcaratus* *Dermacentor andersoni* *Rhipicephalus bursa* and *Argas persicus* adults have resulted in the experimental production of anaplasmosis Transmission by nymphs that had engorged in the larval stage on infected animals and by adults that had similarly engorged as nymphs has been experimentally demonstrated with *Dermacentor albipictus* *D andersoni* *D variabilis* *Ixodes scapularis* *Rhipicephalus bursa* and *R sanguineus* The larval and nymphal progeny of *Hyalomma lusitanicum* removed from an anaplasmosis carrier animal failed to transmit anaplasmosis but the adults of that generation transmitted the disease

It is evident that anaplasmosis transmission by many of the tick species studied is not simply a direct and undelayed mechanical transfer but exhibits features identifying it as biological in nature The etiologic agent is retained and remains alive during the relatively long periods of time required for the stage to stage development of ticks and in some instances is even transmitted by transovarian passage from the adult through the egg to the larva

The question of whether or not the anaplasma bodies leave the erythrocytes after engorgement by the tick to enter its tissue cells or by what other means they may survive until ready for transmission to a new host animal was investigated by Cowdry and Rees[7] Tick species studied by them in this investigation were *Dermacentor albipictus* and *D variabilis* for which stage to stage but not generation to generation transmission has been demonstrated Cowdry and Rees found that in freshly ingested blood the anaplasma bodies showed no evidence of activity within the tick and that they were digested with the erythrocytes that contained them At a time when transmission was presumably possible histological sections revealed no differences between the infected and noninfected ticks

Work reported by Schmidt[8] sheds light on the survival time *in vitro* of *Anaplasma marginale* in carrier blood held for different periods of time at room and at ice box temperatures At the termination of each period 7 cc of the respective sample were inoculated subcutaneously into a susceptible animal Schmidt found the survival periods to be a maximum of 9 days at room temperature and 11 days at ice box temperature

The author[9] studied the survival of the causative agent in Tabanidae In the first trial five *Tabanus sulcifrons* and one *T venustus* that had fully engorged on a carrier animal six days previously were used The abdominal contents were removed and pooled They were crushed with mortar and pestle and then suspended in sterile physiological saline Four cc of this preparation were inoculated subcutaneously into an experimental animal that failed to develop anaplasmosis This animal was later shown by inoculation to be susceptible Fully engorged *Tabanus atratus* specimens were similarly employed at intervals of 6 20 and 48 hours following engorgement Transmission of anaplasmosis resulted only from the 6-hour specimens At the time of preparing these inocula it appeared that digestion of the engorged blood was complete in all but the 6-hour specimens These observations suggest that the causative agent of anaplasmosis is destroyed in Tabanidae by digestion

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by Morris *et al*[16] and Sanders[17] respectively Other flies that have been included in composite experimental feedings with multiple species with positive results by Howell *et al* [18] but have not been individually demonstrated to be vectors are *T fuscirostatus Chrysops sequax* and *Silvius pollinous* *Stomoxys calcitrans* was reported by Sanders[17] to transmit the disease from an animal with clinical anaplasmosis in one experiment that involved direct and undelayed transfer feeding of several hundred specimens

*Transmission by Mosquitoes*

Howell Stiles and Moe[18] reported that mosquitoes are capable of transmitting anaplasmosis under experimental conditions They produced positive transmission to a susceptible animal after composite undelayed transfer feedings from a clinical case with approximately 1500 specimens of *Psorophora columbiae* and *P ciliata* Positive transmission to another susceptible animal resulted following a similar procedure with 241 specimens including *P ciliata P columbiae* and *Aedes aegypti* species The sources of infection in the latter instance included one animal that was recovering from clinical anaplasmosis and one with an active clinical case of the disease The possibility of transmission by mosquitoes from carrier animals was investigated in one instance and transmission did not result For this trial a total of 494 mosquitoes were used including specimens of *P ciliata P columbiae A sigmipennis P cyanescens* and *P discolor*

The authors believe that mosquitoes may at times be responsible for the natural transmission of anaplasmosis that cannot otherwise be logically explained either by mechanical transfer through faulty herd management or by arthropods other than mosquitoes They express the belief that transmission by mosquitoes is dependent upon large numbers and favorable conditions for their feeding Because of their larger size voracious feeding habits and prevalence it is indicated that species of the *Psorophora* genus may be the most important mosquito vectors The authors stress, however that mosquitoes are probably not highly important in spreading anaplasmosis and that they are responsible for active transmission in relatively few instances

*Mechanical Transmission by Manual Manipulation*

Rees[19] demonstrated the ease with which anaplasmosis may be transmitted from infected to susceptible cattle by manual manipulation He was able to transmit the disease with a lancet used first to puncture the ear vein of an infected animal and then for immediate subcutaneous insertion into a susceptible animal It is evident that the amount of blood inoculum necessary to produce anaplasmosis in susceptible animals is extremely small

Present knowledge indicates that the faulty use of surgical or other penetrating instruments is sometimes a most important factor in the seasonal recurrence of anaplasmosis outbreaks In range herd husbandry cattle are commonly corralled in the late winter or early spring months for dehorning castration vaccination blood sampling and similar procedures pertinent to proper care and maintenance If one or more anaplasmosis carrier animals are present in such animal groupings the unintentional transfer of infective blood from carrier to

parts withdrawn blood often exudes from the wound As a result of the pain host animals often make an effort to stop the continued engorgement of the flies by means of switching tail movements and with sweeping head movements over the sides and back which are the most commonly selected engorgement sites The engorgement therefore is often interrupted and the fly must seek a new site to complete its meal The new site may be a new location on the same host but not infrequently is on another animal in the same herd If one or more of the first hosts attacked have *Anaplasma* infected cells circulating in the peripheral blood stream the chances of mechanical transfer of infected erythrocytes on the fly s mouth parts and the inoculation of other animals are greatly increased

Howell *et al* [15] reported positive transmission with several species of *Tabanus* including *americanus oklahomensis sulcifrons abactor equalis erythraeus* and *venustus* They expressed the belief that any species of the genus *Tabanus* is capable of transmitting anaplasmosis by its natural feeding habits if it is active in sufficient numbers They noted that flies that had first partially engorged on recovered carrier animals and then completed feeding on susceptible animals were less efficient as vectors than those that had first fed on animals with clinical cases It was also pointed out that positive tran mission failed to result when transfer feedings between infected and su ceptible animals were interrupted for periods of five minutes or more The smallest number of undelayed trans fer feedings from clinical cases with which the disease was transmitted by the fly vectors used by those workers was 13 by *Tabanus oklahomensis*

The above observations are well substantiated by information included in Dikmans [1] tabulation of the experimental results reported by various workers who have investigated the role of Tabanidae as mechanical vectors In 29 ex periments involving undelayed transfer feedings in whole or in part from ani mals with clinical cases the disease was transmitted in 59 per cent of the trials In 14 experiments using immediate transfer feedings from recovered carrier animals transmission resulted in only 7 per cent of the susceptible cattle When only deferred feedings were employed anaplasmosis was not trans- mitted by flies from either clinical cases or recovered carriers

Tabanidae are variable in size but larger than ticks and mosquitoes and apparently a source of greater discomfort Cattle seem to make greater efforts to prevent their attacks and to displace them from engorgement sites The switching tail and sweeping head movements employed by the host animal to dislodge engorging flies are not very effective in the dislodgement of engorging ticks These latter vectors benefit from a secure dermal attachment and a smaller size that affords great protection in the hair coat of the host Although mosquitoes are less securely attached to the skin during engorgement than ticks their small size also affords considerable protection by the host s hair coat On the basis of the probable relative frequency of interrupted feedings it appears therefore that ticks and very probably mosquitoes are much less important than flies as mechanical vectors

It was mentioned earlier that seven pecies of Tabanidae had been individu ally incriminated as capable experimental mechanical vectors of anapla mosis Two others *Tabanus atratus* and *T fumipennis* have been added to this list

### *Discussion*

Although numerous arthropods have been found capable of transmitting anaplasmosis under experimental conditions the respective roles played by them under natural conditions need further study

Of the 19 species of ticks that have been experimentally incriminated as potential vectors of anaplasmosis in various countries of the world only 6 occur in problematical proportions in the United States Dikmans[8] has evaluated the relative importance of these 6 species on the basis of current experimental and epizootiological knowledge He feels there is convincing and sufficient evidence to support the contention that *Dermacentor occidentalis* and probably *D albipictus* can be considered as vectors of anaplasmosis in nature There is insufficient supportive evidence to be equally positive about *D andersoni D variabilis Ixodes scapularis* and *Argas persicus* at this time

There is sufficient evidence both experimental and epizootiological to identify Tabanidae as important vectors in nature Their efficiency as agents of transmission is not only dependent upon undelayed transfer feedings from infected to susceptible animals but is markedly enhanced when the source of infection is an animal with clinical anaplasmosis rather than a recovered carrier Evidence is too meager to cite the specific role of mosquitoes as vectors in nature but, from all indications they are less important than either ticks or flies

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susceptible animals becomes an unquestioned and distinct possibility This is especially true if operative instruments are not cleansed of blood after the work on each animal is complete When this minimal precaution is ignored it is not uncommon for active clinical cases of anaplasmosis to develop within 30 or more days at a time when horsefly and mosquito populations are beginning their seasonal activity The stage can thus be set for a seasonal outbreak of the disease, the proportions of which will bear a close relation to the incidence of mechanical vectors during that season

Rees and Underwood [9] presented data showing why mechanical vectors such as Tabanidae are more efficient transmitting agents when cattle with active clinical cases rather than carriers are the sources of infection They found that in carrier animals *Anaplasma* infected cells were more prevalent in the bone marrow liver, spleen and lungs and that such cells were rarely observed in the peripheral blood This relation is reversed in the acute case Infected cells are then prevalent in the peripheral blood upon which mechanical vectors so readily feed and relatively scarce in the internal organs

Lotze [1] has reported positive transmission by *Tabanus sulcifrons* from a recovered carrier animal His results suggest an optimal source of infection for transfer by mechanical vectors that merits further investigation The infected animal that Lotze used in this study was one in which *Anaplasma* bodies were found to be microscopically demonstrable in the peripheral blood 11 months after clinical anaplasmosis had occurred Transmission was readily accomplished While it is well known that *Anaplasma* bodies recur in the circulating blood of splenectomized carriers the frequency with which there is such recurrence in intact recovered animals is not known The fact that it might occur in even a very small percentage of carriers however may be important in elucidating the otherwise unexplainable origin of some of the seasonal outbreaks associated with mechanical vectors

## *Transmission* in Utero

It has been indicated that calves occasionally become infected with anaplasmosis *in utero* While it is not known how frequently this occurs it is recognized that such animals presumably remain permanent carriers The author found no evidence that the infection was transmitted *in utero* to any of more than 100 calves secured as experimental animals in a geographical region where the disease is prevalent Most of these calves were subjected to splenectomy without any recurrent manifestations of anaplasmosis and upon inoculation with infective blood all proved susceptible to the disease

Dykstra *et al* [3] made observations on 17 calves from anaplasmosis infected cows All but one of these were free of infection at birth the one exception being a calf that was in the third month of fetal development when its dam had an acute attack of anaplasmosis Of the 16 calves not infected *in utero* 11 were the progeny of cows known to be carriers and 5 were *in utero* during the acute clinical attack of the respective dams A single attempt to transmit anaplasmosis from dam to fetus for two or more generations was accompanied by negative results The evidence suggests that transmission *in utero* is infrequent and a relatively insignificant factor in the propagation of anaplasmosis

# THE PREVENTION AND TREATMENT OF ANAPLASMOSIS

*By* James G Miller

*Department of Pharmacology University of Michigan Ann Arbor Mich*

## *Introduction*

There have been at least 80 drugs proposed for the treatment of anaplasmosis The suggested efficacy of these agents has often been based on a small number of clinical cases with an almost complete lack of prior information as to their specific activity or lack of such activity against *Anaplasma marginale* The host specificity of the parasite has defeated many efforts to evolve a screening method that would adapt itself to the examination of a large number of compounds The previous lack of a preliminary screen has been a major stumbling block in the rational chemotherapeutic approach to the problem

## *Methods of Study*

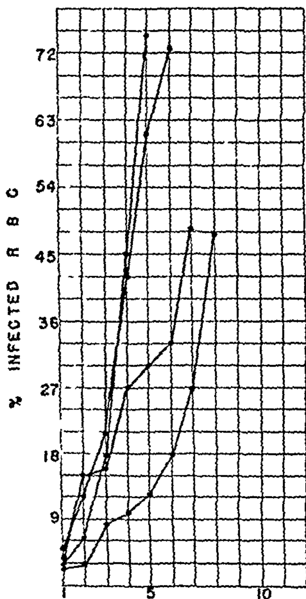
It is difficult to obtain clinical cases of anaplasmosis in ruminants other than the adult bovine and attempts to grow even *Anaplasma marginale* in other hosts or in artificial media have not been successful The role of the spleen in the host s response to anaplasmosis has long been recognized An adult resistant carrier animal will undergo a relapse following splenectomy and calves which at best exhibit only mild symptoms of the disease become susceptible to acute anaplasmosis when splenectomized This has lead to the use of splenectomized calves for the chemotherapeutic screening procedure

In the following experiments male calves were splenectomized between 10 and 14 days of age and then inoculated with *Anaplasma marginale* 2 weeks later The inoculum consisted of a subcutaneous injection of 5 cc of citrated whole blood A common carrier source was used throughout these experiments This carrier was determined[1] to have the most virulent strain of *Anaplasma marginale* available and in fact one of the most virulent that had ever been encountered

Various phases of the disease in splenectomized calves were examined by Lotze[2] and also at Louisiana State University[3] Baton Rouge Louisiana to determine their adaptability to a screening method The incubation period varied widely even when as in these experiments a constant volume of inoculum from a single source was used

It should be pointed out that the incubation period is usually not longer than 60 days and that it can be entirely abolished by massive transfusions of highly infected blood This wide variation in the incubation period makes it an undesirable criterion of drug activity Similarly the peak percentage of infested red blood cells cannot be utilized as it varies widely from animal to animal A peak infestation of 30 per cent of the red blood cells is likely to result in just as severe symptoms and anemia as in the instance of a red cell infestation reaching 85 per cent A splenectomized calf that survives the initial attack of anaplasmosis often undergoes a recurrent series of relapses of varying degrees of severity so that this portion of the disease is not adaptable to the screening

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DAYS FROM 1% INFECTION

[illegible] blood [illegible] by the [illegible]

TABLE 1

VARIATIONS IN THE INCUBATION PERIOD OF 68 SPLENECTOMIZED CALVES*

| Incubation period | No. of animals | Incubation period | No. of animals |
|---|---|---|---|
| 11 days | 1 | 25 days | 3 |
| 12 days | 3 | 26 days | 2 |
| 14 days | 6 | 27 days | 1 |
| 15 days | 4 | 28 days | 2 |
| 16 days | 2 | 29 days | 2 |
| 17 days | 3 | 33 days | 1 |
| 18 days | 3 | 34 days | 1 |
| 19 days | 2 | 37 days | 2 |
| 20 days | 7 | 39 days | 1 |
| 21 days | 6 | 40 days | 1 |
| 22 days | 2 | 41 days | 2 |
| 23 days | 6 | 44 days | 1 |
| 24 days | 4 | | |

Range 11 to 44 days Mean 2[illegible] days Standard deviation ±7 days

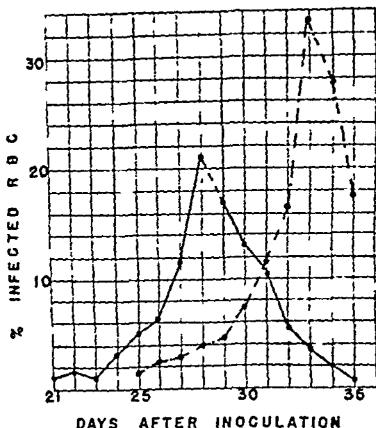
program. The only portion of the disease in splenectomized calves that is consistent enough to be used as a criterion for specific drug activity is the rate of infestation of red blood cells. The percentage of infested red blood cells doubles approximately every 24 hours until a peak is reached (FIGURE 1). For a compound to exhibit specific activity against the *Anaplasma marginale* it would have to alter this pattern of cell infestation.

## *Results*

The drugs previously used in the therapy of this disease can be classified as arsenicals, antimalarials, antimony derivatives and dyes. Two antimalarials, chloroquine dihydrochloride[4] and paludrine diphosphate[5] were subjected to screening with splenectomized calves. In both instances very high dosage levels failed to alter the course of red blood cell infestation (FIGURE 2) and were adjudged to be lacking specific activity against *Anaplasma marginale*.

Chlortetracycline and oxytetracycline were also screened. Chlortetracycline was administered intravenously at the rate of 5 mg./lb. body weight at various stages of erythrocyte infestation. In all instances there was no further increase in the level of infestation following treatment (FIGURE 3). Oxytetracycline gave similar results when administered intravenously at 2.5 mg./lb. body weight. There was 100 per cent recovery in 32 animals treated with these antibiotics as contrasted with 80 per cent fatalities in 24 untreated splenectomized calves.[3] Chloramphenicol therapy resulted in a temporary inhibition of red blood cell infestation which was confined to the period of treatment. Subsequent cell infestation often reached 80 to 90 per cent of all erythrocytes.[6]

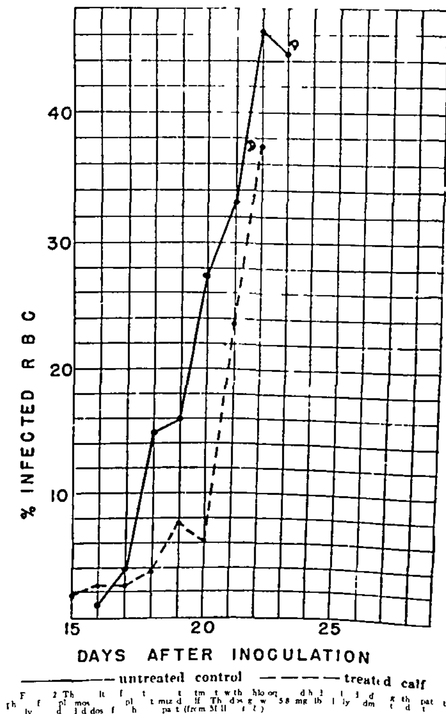
From these experiments it was apparent that chlortetracycline and oxytetracycline had a demonstrable effect on *Anaplasma marginale*. Further experiments were conducted to outline the parameters of this action and its possible utility, if any, in the treatment of this disease. Subinoculations from chlortetracycline and oxytetracycline treated calves indicated that they were still carriers of virulent *Anaplasma marginale*.[3] Several attempts were made to




Fi cau 3 A graph t how th res lt of gl dose of hlort t cy li on the re se of fected d lls The solid line nd t the perc t ge of infected red cells th treated calf The broken portio of that lin ndi tes the day on which chlort t cycl ne wa gi The dotted line nd tes the perc ntage of nfected red cell of the tre ted ontrol calf (from M ue et l )

sterilize carriers of anaplasmosis using large doses of chlortetracycline over a short period of time These experiments failed to rid carriers of the infection[7] in spite of the fact that the blood of these animals was free of infective *Ana plasma marginale* for a brief period following therapy[8] Similar results were obtained when chlortetracycline and oxytetracycline were administered at the rates of 5 and 2 5 mg /lb body weight intravenously for periods of 16 and 20 days respectively[9] Subsequent experiments with both antibiotics at increased dosage levels over a *period of 20 days*[10] *resulted in sterilization of the blood of* carrier animals for at least 100 days and up to 360 days at the last determina tion This was accompanied by the disappearance of *Anaplasma marginale* complement fixing antibodies at an early date following therapy

The possible prophylactic application of these antibiotics was explored Cer tainly if pretreatment could prevent or ameliorate an epizootic a very real ad vance would have been made It soon became apparent however that admin


% INFECTED RBC
10
20
30
40
15
20
25
DAYS AFTER INOCULATION
untreated control
treated calf

and the *Rangelia*) The family name Piroplasmidae was rejected by him on grounds that the genus *Babesia* was created before the genus *Piroplasma* and that according to the nomenclatorial rules the family name should be derived from the oldest named genus in a group of closely allied protozoa The separation of the families was based on the fact that members of the Babesidae multiply within the erythrocytes giving rise to either two or four daughter individuals while those of the Theileridae reproduce by schizogony in the lymphocytes and by binary fission in the erythrocytes

The classification of du Toit (1918) of the species occurring in domestic animals is as follows

Family **BABESIDAE** Poche 1913

In this family are included the nonpigmented parasites of the erythrocytes of mammals which reproduce within these cells by division into two or four daughter individuals. They are transmitted by ticks belonging to the family Ixodidae Murray

Genus **BABESIA** Starcovici 1893

They are mostly of irregular form though double pear shaped forms are observed at a certain stage of the developmental phase Multiplication by binary fission

Subgenus **BABESIA** (Starcovici 1893)

Relatively small and irregularly shaped parasites Average length less than 2 5 microns Multiplication by simple binary fission

*Babesia bovis* (Babes 1888) in cattle

*B ovis* (Babes 1892) in sheep

*B argentina* (Lignieres 1909) in cattle

*B gibsoni* (Patton 1910) in dogs and jackals (*Canis aureus*)

*B divergens* (M Fadyean and Stockman 1911) in cattle

Subgenus **PIROPLASMA** (Patton 1895)

Relatively large binucleated parasites with a small compact and a large less compact nucleus Typically they appear as double pear shaped forms Average length more than 3 0 microns Multiplication into two takes place by a budding process

*Piroplasma bigeminum* (Smith and Kilborne 1893) in cattle

*P canis* Piana and Galli Valerio 1895 in dogs

*P caballi* Nuttall and Strickland 1910 in solipeds

*P trautmanni* Knuth and du Toit 1918 in pigs

Genus **NICOLLIA** Nuttall 1908

Distinctly binucleated oval or pear shaped parasites Division into four daughter cells

*Nicollia quadrigemina* (Nicolle 1907) in rodents (*Ctenodactylus gondi*) This genus is not described in domestic animals

# CLASSIFICATION, TRANSMISSION, AND BIOLOGY OF PIROPLASMS OF DOMESTIC ANIMALS

*By* W. O. Neitz

*Department of Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Union of South Africa*

## Discussion on the Classification of the Piroplasms

Attempts at classifying the piroplasms of domestic animals date back to the time when Babes discovered *Babesia bovis* (Babes 1888) and *B. ovis* (Babes 1892) in Romania. Smith and Kilborne found *Piroplasma bigeminum* (Smith and Kilborne 1893) in cattle in the United States of America and Koch (1898) described the infectious agent of East Coast fever of cattle in Tanganyika, Africa. It is interesting to note that both Koch (1898) and Theiler (1904) included the responsible parasite in the genus *Piroplasma*. The former investigator named the protozoon *Piroplasma bacilliformis* while the latter assigned the name *Piroplasma parvum* to it. A second parasite of cattle, the erythrocytic stages of which resembled *Piroplasma parvum* was found by Theiler (1906b) in South Africa. It soon became evident that the latter two parasites could not be retained in the genus *Piroplasma* and therefore Bettencourt, França and Borges (1907) transferred them to a new genus *Theileria*. As no schizonts (Koch bodies) had been observed in the developmental cycle of *Theileria mutans*, du Toit (1918) placed it in the genus *Gonderia*. After it had been established by several investigators in Africa that Koch bodies do occur in the life cycle of *Gonderia mutans*, Theiler and Graf (1928) retransferred this protozoon to the genus *Theileria*. From these brief introductory remarks it becomes evident that a great deal of confusion existed in the nomenclature of this important group of organisms. The writer does not hesitate to say that much work will still have to be done before the classification and the nomenclature of the piroplasms can be placed on a sound basis.

Du Toit (1918) and Thomson and Hall (1933) have given the following brief review on the classification of the piroplasms. França (1910) created the family Piroplasmidae into which he placed five genera, namely *Piroplasma* Patton 1895, *Theileria* Bettencourt, França and Borges 1907, *Nicollia* Nuttall 1908, *Nuttallia* França 1910b, and *Smithia* França 1910. In 1917 and 1918 França revised this classification and included the genera *Achromaticus* Dionisi 1899a, b, *Elleipsisoma* França 1912, *Paraplasma* Seidelin 1917, *Rangelia* Carini and Maciel 1914 and *Rossiella* Nuttall 1912a into the above mentioned family.

The first systematic study was that of du Toit (1918) who modified França's scheme. Du Toit rejected the genera *Achromaticus*, *Elleipsisoma* and *Paraplasma* on grounds that they are not piroplasms. He retained two families, the Babesidae Poche 1913 comprising seven genera (*Babesia* Starcovici 1893, *Piroplasma*, *Nuttallia*, *Nicollia*, *Smithia*, *Rossiella* and *Gonderia* du Toit 1918) and the family Theileridae du Toit 1918 comprising two genera (*Theileria*

and the *Rangelia*) The family name Piroplasmidae was rejected by him on grounds that the genus *Babesia* was created before the genus *Piroplasma* and that according to the nomenclatorial rules the family name should be derived from the oldest named genus in a group of closely allied protozoa The separation of the families was based on the fact that members of the Babesidae multiply within the erythrocytes giving rise to either two or four daughter individuals while those of the Theileridae reproduce by schizogony in the lymphocytes and by binary fission in the erythrocytes

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Genus **BABESIA** Starcovici 1893

They are mostly of irregular form though double pear shaped forms are observed at a certain stage of the developmental phase Multiplication by binary fission

Sub genus **BABESIA** (Starcovici 1893)

Relatively small and irregularly shaped parasites Average length less than 2 5 microns Multiplication by simple binary fission

*Babesia bovis* (Babes 1888) in cattle
*B ovis* (Babes 1892) in sheep
*B argentina* (Lignières 1909) in cattle
*B gibsoni* (Patton 1910) in dogs and jackals (*Canis aureus*)
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*P canis* Piana and Galli Valerio 1895 in dogs
*P caballi* Nuttall and Strickland 1910 in solipeds
*P trautmanni* Knuth and du Toit 1918 in pigs

Genus **NICOLLIA** Nuttall 1908

Distinctly binucleated oval or pear shaped parasites Division into four daughter cells

*Nicollia quadrigemina* (Nicolle 1907) in rodents (*Ctenodactylus gondi*) This genus is not described in domestic animals

Genus **NUTTALLIA** França 1909

Oval or pear shaped parasites that divide into four, producing the cross arrangement No typical rod forms are seen

*Nuttallia equi* (Laveran 1901) in solipeds

*Nuttallia* sp (Dschunkowsky and Luhs 1909) in sheep in Transcaucasia

*N asini* (Dschunkowsky and Luhs 1913) in donkeys

*Nuttallia* sp (Dschunkowsky and Luhs 1913) in horses in Transcaucasia

Genus **SMITHIA** França 1910

Parasites that are pear shaped and stretch across the cell Division in the cross form

*Smithia microti* (França 1910) in the field mouse

*S talpae* (Galli Valerio 1913) in the mole This genus is not described in domestic animals

Genus **ROSSIELLA** Nuttall 1912

Parasites are large and rounded in shape One to four parasites occur in an erythrocyte

*Rossiella rossi* (Nuttall 1910) in the jackal (*Canis adustus*) This genus is not described in domestic animals

Genus **GONDERIA** du Toit 1918

Parasites are round oval or rod shaped Division into four in the cross arrangement The daughter forms are composed mostly of chromatin

*Gonderia mutans* (Theiler 1906b) in cattle

*G hirci* (Dschunkowsky and Luhs 1909) in goats

*G buffeli* (Neveu Lemaire 1912) in buffaloes

*G camelensis* (Yakimoff and Schokhor 1917) in camels

Family **THEILERIDAE** du Toit 1918

Endoglobular nonpigmented blood parasites which are transmitted by ticks belonging to the family Ixodidae Multiplication chiefly in the cells of the lymphatic system

Genus **THEILERIA** Bettencourt França and Borges 1907

Small round or rod shaped parasites Multiplication by division into two in the red blood cells or by schizogony in the cells of the lymphatic system

*Theileria parva* (Theiler 1904) in cattle

*Th annulata* (Dschunkowsky and Luhs 1904) in cattle

*Th ovis* (Littlewood 1915) in sheep

Genus **RANGELIA** Carini and Maciel 1914

Parasites are round oval or pear shaped Multiplication by division into two in the red blood cells or by schizogony in the endothelial cells

*Rangelia vitalli* (Pestana 1910) in dogs

Wenyon (1926) critically reviewed and completely revised du Toit s classification In the family Babesidae he discarded the separate generic names *Piroplasma Nicollia Nuttallia Smithia Rossiella* and *Gonderia* as well as the

generic names *Babesiella* of Mesnil (1919) and *Microbabesia* of Sohns (1918) and substituted the single genus *Babesia* In the family Theileridae he retained the single genus *Theileria* and rejected the generic name *Rangelia* (*Rangelia vitalli* Pestana 1910) on the grounds that there is insufficient evidence to justify differentiation of this parasite from *Babesia canis* (Piana and Galli Valerio 1895) He regarded the schizonts of *R vitalli* as in reality nothing other than *Toxoplasma* or phagocytosed organisms

Wenyon s revised classification of the piroplasms is as follows

Suborder **PIROPLASMIDEA** Wenyon 1926

In this suborder are included certain parasites which inhabit red blood corpuscles of mammals but do not form the pigment (hemozoin) characteristic of members of the genera *Haemoproteus* Kruse 1890 and *Plasmodium* Marchiafava and Celli 1885 Each parasite consists of a minute portion of cytoplasm and a nucleus and in dry films stained by Romanowsky stains it appears as a blue staining cytoplasm and a red staining chromatin portion consisting frequently of a red granule with a string of finer granules extending from it When a vacuole is present in the cytoplasm as is not infrequently the case it may be difficult to distinguish from a young ring form of a malarial parasite Whereas the majority of the malarial parasites reproduce by a process of schizogony and give rise to a fairly large number of merozoites the piroplasmata reproduce by a division into only two or four daughter individuals However certain members of the genus *Plasmodium* as for instance *P minasense* Carini and Rudolph 1912 of lizard divide into or rather bud off four merozoites only and were it not for the pigment which is present it would be impossible to distinguish these from certain piroplasmata such as *Babesia quadrigemina* and *Achromaticus vesperuginis* Dionisi 1899 which reproduce in a similar manner These forms appear to stand as a connecting link between the Haemosporidiidea and the Piroplasmidea (The exoerythrocytic schizonts have not yet been demonstrated in *Pl minasense*)

A parasite of cattle (*Theileria parva*) the cause of East Coast fever differs from other piroplasmata in that a definite schizogony process occurs not in the erythrocytes but in internal organs within the cells of the lymphocytic series The schizonts (Koch blue bodies) produce a large number of merozoites and on this account it is impossible to group this form with the members of the genus *Babesia* which reproduce only in the red blood corpuscles

Family **BABESIDAE** Poche 1913

Genus **BABESIA** Starcovici 1893

Synonyms *Piroplasma* *Nicollia* *Nuttallia* *Rossiella* *Smithia* *Gonderia* *Babesiella* *Microbabesia* and *Achromaticus*

Family **THEILERIDAE** du Toit 1918

Genus **THEILERIA** Bettencourt França and Borges 190

Genus **RANGELIA** C  ini and Maciel 1914

Regarded as an invalid genus

This classification based on essential morphological characteristics was

accepted by Doflein and Reichenow (1929) and by Thomson and Hall (1933) but not by Donatien and Lestoquard (1930), nor by Yakimoff (1931) Wenyon s scheme has nevertheless reduced to simplicity the rather confused nomenclature of the piroplasms

In adopting the single genus *Babesia* in the family Babesidae Wenyon (1926) drew attention to the fact that both in cattle and in sheep the parasites could be separated on the basis of size into three groups namely large intermediate and very small forms The species in cattle became *Babesia bigemina* (Smith and Kilborne 1893) (largest form) *B bovis* (Babes 1888) (intermediate form) and B *mutans* (Theiler 1906b) (smallest form) The species in sheep were named *Babesia motasi* Wenyon 1926 the largest form *B ovis* (Babes 1892) the intermediate form and *B sergenti* Wenyon 1926 the smallest form Subsequent studies by Theiler and Graf (1928) and Lestoquard (1929) however on the smallest forms in cattle and sheep in which schizonts were eventually found showed that these parasites could no longer be retained in either the genus *Babesia* or *Gonderia* These investigators transferred the parasites to the genus *Theileria*

Donatien and Lestoquard (1930) reviewed the classification of the piroplasms of domestic animals and disagree entirely with Wenyon s rejection of certain genera They adopt two families, namely the Piroplasmidae and the Theileridae and are of the opinion that on morphological biological epizootiological and pathological characteristics three genera should be retained in the family Piroplasmidae for the piroplasms of cattle sheep horses dogs and pigs

The classification of Donatien and Lestoquard is as follows

Family PIROPLASMIDAE França 1910

Genus PIROPLASMA Patton 1895

Subgenus PIROPLASMA (Patton 1895)

*Piroplasma bigeminum* in cattle
*P ovis* in sheep
*P caballi* in horses
*P canis* in dogs
*P trautmanni* in pigs

Subgenus BABESIELLA Mesnil 1919

*Babesiella bovis* in cattle
*B argentina* in cattle
*B berbera* in cattle
*B major* in cattle
*B ovis* in sheep

Genus NUTTALLIA França 1910

*Nuttallia equi* in solipeds

Family THEILERIDAE du Toit 1918

Genus THEILERIA Bettencourt França and Borges 1907

*Theileria parva* in cattle
*Th mutans* in cattle

*Th dispar* in cattle
*Th o is* in sheep and goats (pathogenic)
*Th recondita* in sheep and goats (nonpathogenic)

In the above classification Donatien and Lestoquard (1930) contend that size morphology and position of the parasites in the erythrocytes justify the retention of these genera On this basis and on the absence of cross immunity in two of the species they conclude that actually four *Babesia* species occur in cattle These are differentiated as follows

*Babesiella bovis* (Babes 1888) Parasites appear as pear shaped (1.5 microns to 2 0 microns in length) or amoeboid forms (1 0 microns to 1 5 microns in diameter) They are commonly situated on the periphery of erythrocytes Angle of divergence between two pear shaped individuals is obtuse

*Babesiella argentina* (Lignières 1909) resembles *B bovis* very closely, but differs from the latter in that it is chiefly situated centrally

*Babesiella berbera* Sergent Donatien Parrot Lestoquard Plantureux and Rougebief 1924b possesses the same features as *B argentina* but can be differentiated from it by the absence of a cross immunity

*Babesiella major* Sergent Donatien Parrot Lestoquard and Plantureux 1926 It is relatively larger than *B bovis* The diameter of the round forms is 1 8 microns and the length of the pear shaped form is 2 6 microns These organisms are situated centrally in the erythrocytes

Thomson and Hall (1933) agree with Donatien and Lestoquard (1930) that the various morphological differences such as size and shape and mode of reproduction justify the differentiation into species in cattle namely *Babesia (Piroplasma) bigemina* and *Babesia (Babesiella) bovis* On morphology alone it is impossible to differentiate *B argentina B berbera* and *B major* from *B bovis* Richardson (1948) contends that absence of cross immunity between *B berbera* and *B argentina* which resemble each other morphologically cannot be used as a basis of differentiation between species He is of the opinion that *B berbera* bears the same relationship to *B argentina* as one strain of *B canis* does to another (it has been shown that immunologically different strains occur in *B canis*) Furthermore Richardson states that on this ground *B berbera* should be considered as an African strain of *B argentina*

Donatien and Lestoquard (1930) stress that trypan blue is a specific in the treatment of *Piroplasma* infections but has absolutely no action on the *Babesiella* species On this feature alone they maintain that there is sufficient reason for retaining separate generic names

In his attempt at classifying the piroplasms of cattle Yakimoff (1931) modified the scheme of Donatien and Lestoquard (1930) He disagrees with the views of the French worker as far as the efficacy of trypan blue is concerned in the treatment of bovine babesiellosis but accepts the difference between the immunological properties possessed by the *Babesiella* species as a ba is for differentiation between species He concludes from his experience that trypan blue is specific for *Babesiella bo is* and not for the other *Babesiella* species *B bovis* (Babes 1888) is placed in the subgenus *Babesiella* The remaining *Babesiella* species are transferred to the ubgenus *Francaiella* The species of the latter subgenus are divided into two types namely small and large

Yakimoff differentiates between the species of each type on grounds that each of them possesses distinct immunological properties

Yakimoff's classification of the piroplasms in cattle is as follows

Genus **PIROPLASMA** Patton 1895

Unpigmented endoglobular parasites Besides the pear shaped forms which often occur in pairs there are ring and elliptical forms This genus can be divided into two subgenera

Subgenus **PIROPLASMA** s str

The typical forms are pear shaped often occur in pairs and are situated centrally in the erythrocytes The angle of divergence between two individuals is always acute The length of these forms is more than half the diameter of the erythrocytes and harbor no less than two chromatin masses Round forms may also be seen Trypan blue is specific

*Piroplasma bigeminum*

Subgenus **BABESIELLA** Mesnil 1919

Pear shaped ring and elliptical forms The length of the pear shaped forms is equal to or less than that of the radius of the erythrocytes The angle of divergence between two individuals is usually obtuse Parasites are situated peripherally or centrally One chromatin mass is seen in the pear shaped forms Round forms are more common than pear shaped forms Trypan blue is not always effective This subgenus can be divided into two groups

Group **BABESIELLA** s str

Pear shaped ameboid or ring forms which are situated peripherally in the erthrocytes Length of the pear shaped forms less than half the diameter of the erythrocytes Number of parasitised red blood cells may be very large Trypan blue is specific

*Babesiella bovis*

Group **FRANCAIELLA** Yakimoff 1926

Pear shaped ring and ameboid forms are situated centrally in the erythrocytes Length of the pear shaped forms is equal to or less than half the diameter of the erythrocytes Percentage of parasitised erythrocytes is small Trypan blue is not specific

Type 1 Length of parasite smaller than that of radius of erythrocytes *Francaiella caucasica F occidentalis*

Type 2 Length of parasite the same as that of radius of erythrocytes *Francaiella major F colchica*

Consideration of Yakimoff's classification clearly indicates that his scheme cannot be readily applied in practice In some if not in all cases a diagnosis would involve conducting treatment with trypan blue in order to determine the subgenus and undertaking a series of cross immunity tests for establishing the species

The writer has discussed the scheme on the classification of the *Babesiella*

species as indicated by the French and Russian investigators with L C Newton from Australia I Tzur from Israel and James Carmichael and U F Richardson from England and agrees with them that studies on the morphological biological epidemiological pathological and chemotherapeutical characteristics of these protozoa should be conducted at a central laboratory The results of such investigations will undoubtedly establish whether these protozoa are distinct species or simply strains of *B bovis* varying in virulence and immunogenicity

Another contribution to the classification of the piroplasms is that of Neitz and Steyn (1947) who revised the nomenclature of the canine *Babesia* species Until 1938 it was generally accepted that *B canis* is specific for the dog This conception had to be abandoned when Schoop and Dedie (1938) demonstrated that the silver fox is susceptible to *B canis* This observation naturally suggested that further work should be undertaken in order to establish whether or not the conclusions of Lounsbury (1903a) Nuttall and Graham Smith (1909) and Mettam (1933) regarding the insusceptibility of two species of African jackals (*Thos* species) to *B canis* were justified Neitz and Steyn (1947) successfully transmitted *B canis* to and from susceptible splenectomized jackals back into dogs without any difficulty From this they concluded that *B canis* is not a monoxenous protozoon but like *B gibsoni* a stenoxenous parasite This observation also permitted them to revise the nomenclature of the canine *Babesia* species as indicated below

For the sake of completeness it is necessary to add the views of Reichenow about the *Babesia* species occurring in dogs He concludes from the tick transmission experiments conducted by Nieschulz and Wawo Roentoe (1934) and those undertaken by himself (Reichenow 1935) that several distinct species occur in nature He bases the differentiation on the vector specificity and on the absence of a cross immunity between the parasites transmitted by them The name *B canis* he retains for the parasite transmitted by the tick *Dermacentor marginatus* and assigns the name *B vogeli* (Reichenow 1937) for the protozoon transmitted by *Rhipicephalus sanguineus* He also states that *B vogeli* is larger and less pathogenic than *B canis* Furthermore he suggests that *B canis* which is transmitted by *Haemaphysalis leachi* in South Africa is in all probability yet another distinct species Enigk (1944b) on the contrary comes to the conclusion that vector specificity cannot be used as a basis for the differentiation between species He showed that the *B canis* strain that he obtained from W Kikuth (Elberfeld Germany) can be transmitted by *Rhipicephalus sanguineus Dermacentor pictus* and *Hyalomma marginatum* The inability of transmitting *P canis* by certain ticks is obviously attributable to as yet unknown factors

The classification of Neitz and Steyn (1947) of the *Babesia* species of the family Canidae is as follows

Family **BABESIDAE** Poche 1913

*Babesia canis* (Piana and Galli Valerio 1895)

Synonyms *Piroplasma canis* (Piana and Galli Valerio 1895) *Piroplasma rossi* Nuttall 1910 *Rossiella rossi* (Nuttall 1910) *Babesia rossi* (Nuttall 1910)

*Babesia gibsoni* (Patton 1910)

Synonyms *Piroplasma gibsoni* Patton 1910 *Theileria* species Yakimoff and Schokhor 1917 *Nuttallia baueryi* Leger and Bedier 1922

*Babesia vogeli* (Reichenow 1937)

Synonym *Babesia major* Reichenow 1935

The last contribution on the revision of the classification of the group of piroplasms commonly referred to as Theilerias has recently been submitted by Neitz and Jansen (1956) for publication in the Onderstepoort Journal These workers have come to the conclusion that the Theilerias can no longer be retained in the suborder Piroplasmidea Before continuing with the revised classification of the Theilerias the salient features arising out of the discussion on the *Babesia* species as well as the classificatory list of these protozoa will be given first

## Conclusions Regarding the Classification of the *Babesia* Species

Having surveyed in detail the complicated history of the nomenclature of the *Babesia* species and having indicated the criteria used by Wenyon (1926) Thomson and Hall (1933) and Neitz and Steyn (1947) for what appears to be a more satisfactory basis for their classification the revised classification is presented below in summary form For the sake of completeness the generic and specific names as well as the generic and specific synonomy as listed by França (1918) du Toit (1918) Wenyon (1926) Donatien and Lestoquard (1930) Yakimoff (1931) Neveu Lemaire (1943) Neitz and Steyn (1947) and Doflein and Reichenow (1953) are included It is self evident that this scheme will have to be revised as our knowledge on the life cycles of these protozoa in both the vertebrate and invertebrate hosts and of their biological properties is worked out in detail In this connection it should be mentioned that Reichenow (1938) has suggested that the *Babesia* species bear some resemblance to the *Amoeba* and that they may in future be included in the class Rhizopoda

The classification of the *Babesia* species is as follows

Suborder **PIROPLASMIDEA** Wenyon 1926

Family **BABESIDAE** Poche 1913

Genus **BABESIA** Starcovici 1893

Synonyms *Piroplasma* Patton 1895 *Achromaticus* Dionisi 1899 *Nicollia* Nuttall 1908 *Nuttallia* França 1909 *Smithia* França 1909 *Rossiella* Nuttall 1912 *Microbabesia* Sohns 1918 *Gonderia* du Toit 1918 *Babesiella* Mesnil 1919 *Francaiella* Yakimoff 1926

*Species of* Babesia *in cattle*

*Babesia bovis* (Babes 1888)

Synonyms *Haematococcus bovis* Babes 1888 *Babesia bovis* Starcovici 1893 *Piroplasma bovis* Patton 1895 *Piroplasma divergens* M Fadyean and Stockman 1911 *Microbabesia divergens* Sohns 1918 *Babesiella bovis* Mesnil 1919

*Babesia bigemina* (Smith and Kilborne 1893)
Synonyms *Pyrosoma bigeminum* Smith and Kilborne 1893 *Apiosoma bigeminum* Vandolleck 1895 *Piroplasma bigeminum* Patton 1895 *Piroplasma australe* Miranda and Parreiras Horta ? *Babesia hudsonius bovis* Bowhill 1909

*Babesia argentina* (Lignières 1909)
Synonyms *Piroplasma argentinum* Lignières 1903, *Francaiella argentina* Yakimoff 1926

*Babesia berbera* Sergent Donatien Parrot Lestoquard Plantureux and Rougebief 1924a b
Synonyms *Francaiella caucasica* Yakimoff and Bélawine 1926 ? *Francaiella occidentalis* Yakimoff and Bourzeff 1927

*Babesia major* Sergent Donatien Parrot Lestoquard and Plantureux 1926
Synonym *Francaiella colchica* Yakimoff 1928

*Species of* Babesia *in sheep and goats*

*Babesia motasi* Wenyon 1926
Synonyms *Haematococcus ovis* Babes 1892 part *Piroplasma ovis* Lestoquard 1925

*Babesia ovis* (Babes 1892)
Synonyms *Haematococcus ovis* Babes 1892 part *Babesia ovis* Starcovici 1893 *Piroplasma ovis* Laveran and Nicolle 1899 *Piroplasma hirci* Dschunkowsky and Luhs 1909 *Babesiella ovis* Lestoquard 1924

*Species of* Babesia *specific for sheep*

*Babesia foliata* Ray and Rhaghavachari 1941

*Species of* Babesia *so far recorded only in a goat*

*Babesia taylori* (Sarwar 1935)
Synonym *Piroplasma taylori* (Sarwar 1935)

*Species of* Babesia *in members of the family Equidae (horses etc)*

*Babesia caballi* (Nuttall 1910)
Synonym *Piroplasma caballi* Nuttall 1910

*Babesia equi* (Laveran 1901)
Synonyms *Piroplasma equi* Laveran 1901 *Nuttallia equi* Laveran 1901 *Nuttallia asini* Dschunkowsky and Luhs 1913 ? *Nuttallia minor* Sassuchin 1933

*Species of* Babesia *in members of the family Canidae (dog jackal etc.)*

*Babesia canis* (Piana and Galli Valerio 1895)
Synonyms *Pyrosoma bigeminum* var *canis* Piana and Galli Valerio 1895 *Piroplasma canis* (Piana and Galli Valerio 1895) *Babesia rossi* Nuttall 1910 *Rossiella rossi* Nuttall 1912

*Babesia vogeli* (Reichenow 1937)
Synonym *Babesia major* Reichenow 1935

*Babesia gibsoni* (Patton 1910)

Synonyms *Piroplasma gibsoni* Patton, 1910, *Theileria* species Yakimoff and Schokhor 1917 *Nuttallia bauryi* Leger and Bedier 1922

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Synonym *Babesia major* Reichenow 1935

The last contribution on the revision of the classification of the group of piroplasms commonly referred to as Theilerias has recently been submitted by Neitz and Jansen (1956) for publication in the Onderstepoort Journal These workers have come to the conclusion that the Theilerias can no longer be retained in the suborder Piroplasmidea Before continuing with the revised classification of the Theilerias the salient features arising out of the discussion on the *Babesia* species as well as the classificatory list of these protozoa will be given first

## Conclusions Regarding the Classification of the *Babesia* Species

Having surveyed in detail the complicated history of the nomenclature of the *Babesia* species and having indicated the criteria used by Wenyon (1926) Thomson and Hall (1933) and Neitz and Steyn (1947) for what appears to be a more satisfactory basis for their classification the revised classification is presented below in summary form For the sake of completeness the generic and specific names as well as the generic and specific synonomy as listed by França (1918) du Toit (1918) Wenyon (1926) Donatien and Lestoquard (1930) Yakimoff (1931) Neveu Lemaire (1943) Neitz and Steyn (1947) and Doflein and Reichenow (1953) are included It is self evident that this scheme will have to be revised as our knowledge on the life cycles of these protozoa in both the vertebrate and invertebrate hosts and of their biological properties is worked out in detail In this connection it should be mentioned that Reichenow (1938) has suggested that the *Babesia* species bear some resemblance to the *Amoeba* and that they may in future be included in the class Rhizopoda

The classification of the *Babesia* species is as follows

Suborder **PIROPLASMIDEA** Wenyon 1926

Family **BABESIDAE** Poche 1913

Genus **BABESIA** Starcovici 1893

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*Species of* Babesia *in cattle*

*Babesia bovis* (Babes 1888)

Synonyms *Haematococcus bovis* Babes 1888 *Babesia bovis* Starcovici 1893 *Piroplasma bovis* Patton 1895 *Piroplasma divergens* M Fadyean and Stockman 1911 *Microbabesia divergens* Sohns 1918 *Babesiella bovis* Mesnil 1919

other cattle and by the fact that ticks may still infect themselves It should be mentioned at this stage that the development of a sterile immunity following recovery from East Coast fever is based on field observations made by Theiler (1921) The latter s conclusion was subsequently confirmed experimentally by du Toit (1928) and by Neitz (1948)

The comments of Wenyon (1926) on the behavior of the erythrocytic stages of *Th parva* and *Th mutans* are significant if one considers the basis on which the family Plasmodiidae Mesnil 1903 is differentiated from the family Haemoproteidae Doflein 1916 In the former the schizogony occurs either in the endothelial cells lining the blood capillaries or in the hepatic cells as well as in the erythrocytes which also harbor gametocytes In the Haemoproteidae the schizogony cycle occurs in the endothelial cells lining the blood capillaries and the forms that appear in the red blood cells are gametocytes A similar difference exists between the life cycles of *Th parva* and *Th mutans* and hence it is believed that this justifies a revision of the classification of those theilerias of which the vertebrate cycle of development is known The hope is expressed that this amendment will lead to a clearer understanding of the infectious agents responsible for East Coast fever and allied diseases

The generic and specific nomenclatures of the *Theileria* species have undergone several changes Originally the first three distinct species described from cattle were included in the genus *Piroplasma* Patton 1895 and were named *P kochi* Stephens and Christophers 1903 (= *P parvum* Theiler 1904) *P annulatum* Dschunkowsky and Luhs 1904 and *P mutans* Theiler 1906b Bettencourt França and Borges (1907) compared the life cycle and morphology of *P bigeminum* Smith and Kilborne 1893 with that of *P parvum* and *P annulatum* and concluding that the presence of schizonts in the developmental cycle of the latter two protozoa justified their removal from the genus *Piroplasma* placed them in a new genus *Theileria* The organisms were renamed *Theileria parva* and *Theileria annulata* C França (1909) transferred *P mutans* to the genus *Theileria* even though he was aware that schizonts had not been demonstrated in the life cycle of this protozoon He considered that the resemblance that exists between the erythrocytic stages of *P mutans* and *Th parva* was sufficient reason for changing the generic name França modification of the nomenclature was not generally accepted Theile Gray and Power (1914) who had seen the cross forms in this parasite suggested that it would be more reasonable to include *P mutans* in the genus *Nuttallia* França 1910 in which the same mode of multiplications occurs

From this brief historical review it becomes apparent that no less than four generic names namely *Piroplasma* *Theileria* *Gonderia* and *Babesia* had been assigned to the protozoon *Piroplasma mutans* Brumpt (1923) concludes from circumstantial evidence that Theiler s species of *Piroplasma* belongs to the genus *Theileria* No convincing evidence was available for the validity of any of the above mentioned genera until Viljoen (1923) Martinaglia (1924) Viljoen and Martinaglia (1928) Doyle (1924) and Curson (1928) found schizonts in calves harboring *Gonderia mutans* These observations prompted Theiler and Graf (1928) to study the life cycle of this protozoon more closely In doing so they found Koch bodies in several calves and concluded that

*Babesia gibsoni* (Patton, 1910)

Synonyms *Piroplasma gibsoni* Patton 1910 *Achromaticus gibsoni* França 1917 *Babesiella gibsoni* Mesnil, 1919 *Nuttallia bauryi* Leger and Bedier 1922

*Species of* Babesia *in swine*

*Babesia trautmanni* (Knuth and du Toit 1918)

Synonyms *Piroplasma trautmanni* Knuth and du Toit 1918 *Piroplasma suis* Lanzillo, 1924

*Babesia perroncitoi* (Cerruti 1939)

Synonym *Babesiella perroncitoi* Cerruti 1939

*Species of* Babesia *in members of the family Felidae (domestic and wild cats)*

*Babesia felis* Davis 1929

Synonyms *Babesiella felis* Carpano 1934 *Nuttallia felis* var *domestica* Jackson and Dunning 1937

The names assigned to members of a certain group of protozoa at present included in the genus *Theileria* have long been the subject of discussion much of which has hopelessly intermixed zoological and nomenclatorial considerations In a general review on the classification of the piroplasms Thomson and Hall (1933) mention that in the present state of our knowledge it would seem that there is a general agreement that there is only a single genus in the family Theileridae namely *Theileria*

Neitz and Jansen (1956) carefully studied the paper of Thomson and Hall, and arrived at the conclusion that in the past various workers (Bettencourt França and Borges 1907 du Toit 1918 Theiler and Graf 1928 Sergent Donatien Parrot and Lestoquard 1929 Donatien and Lestoquard 1930) believed that the presence of schizonts (Koch bodies) determined their inclusion in the genus *Theileria* In doing so little attention was paid to the erythrocytic stages of these parasites even though Wenyon (1926) pointed out that in contradistinction to *Babesia mutans* (= *Piroplasma mutans* = *Theileria mutans*) the endoglobular stage of *Theileria parva* does not reproduce In support of his assertion he states that although the erythrocytic stages may sometimes be seen in pairs or occasionally in fours as in the cross forms it is doubtful if these represent divisional stages as they do in the case of *B mutans* the morphological resemblance to which may be very striking Actual division of *Th parva* was never observed to take place in the living condition though in stained films parasites which might be interpreted as in the process of division were sometimes seen In *Th parva* inoculation of blood will not as a rule convey infection so that it is presumed that blood forms represent gametocytes which are destined to develop in the tick If animals recover from the disease (East Coast fever) the parasites disappear from the blood and this disappearance is absolute for ticks can no longer be infected from them In this respect again *Th parva* differs from the species of *Babesia* (= *Th mutans*) which though disappearing microscopically are still present for years after clinical recovery as proved by the infectivity of blood on direct inoculation of

other cattle and by the fact that ticks may still infect themselves It should be mentioned at this stage that the development of a sterile immunity following recovery from East Coast fever is based on field observations made by Theiler (1921) The latter's conclusion was subsequently confirmed experimentally by du Toit (1928) and by Neitz (1948)

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*Gonderia mutans* should be named *Theileria mutans* and that the genus *Gonderia* should be sunk in favor of the genus *Theileria*. This suggestion was accepted by Sergent, Donatien, Parrot and Lestoquard (1929) who also encountered Koch bodies in cattle infected with *Theileria mutans*. Lestoquard (1929) demonstrated schizonts in splenectomized sheep and goats harboring *Gonderia ovis* (= *Babesia sergenti* Wenyon, 1926) after the intravenous administration of cultures of paratyphoid B. From this observation he deduced that *Gonderia ovis* Lestoquard, 1924, belongs to the genus *Theileria* and he proposed the name *Theileria recondita*.

A complete historical review of the literature dealing with theileriosis in cattle and sheep has been provided by du Toit (1930). This contribution has done much to help workers to a clearer understanding of this important group of protozoa.

The description of *Theileria parva* by Theiler (1904), of *Theileria annulata* by Dschunkowsky and Luhs (1904) and of *Theileria mutans* by Theiler (1906b) was followed by studies on this group of organisms in many parts of the world. A fourth species in cattle, *Theileria dispar* Sergent, Donatien, Parrot, Lestoquard, Plantureux and Rougebief, 1924a, b, was described in Algeria, and a fifth species in the buffalo and in cattle. Two parasites named *Theileria sergenti* Yakimoff and Dekhtereff, 1930, and *Theileria (Gonderia) orientalis* (Yakimoff and Sondatschenkow, 1931) have been described from cattle in Asia. It has not been possible to determine the validity of either species. They may be synonymous for either *Th. mutans* or *Th. annulata*.

The question is discussed by du Toit (1930) as to whether all cases of theileriosis in cattle that have been described in the literature fit into the four types mentioned above. Du Toit comes to the conclusion that there are intermediate stages between these forms. Altogether there are at least eight types of theileriosis in cattle that can be distinguished. These he arranged in the order of virulence as follows:

(1) *Theileria parva*. South Africa. Mortality of 90 per cent to 100 per cent. Koch bodies are constantly present.

(2) Theileriosis (Amakebe). East Africa. The virulence is low. Mortality of about 15 per cent.

(3) *Theileria dispar*. Algeria. Mortality of 20 per cent to 90 per cent.

(4) Theileriosis. Morocco. A chronic or mild infection capable of becoming acute if animals are subjected to adverse conditions.

(5) *Theileria annulata*. Tunis, Asia Minor, Russia. Mortality of 5 per cent to 20 per cent and sometimes even greater.

(6) Theileriosis (a) North Eastern Rhodesia. Koch bodies found (Turnbull, 1926). (b) India. Koch bodies found (Cooper, 1926). (c) Egypt. Koch bodies found (Doyle, 1924).

(7) *Theileria mutans*. South Africa and Algeria. Strains with Koch bodies.

(8) *Theileria mutans*. South Africa and Algeria. Strains without Koch bodies.

In (7) and (8), 999 cases out of 1,000 produce no symptoms or very slight anemia. Under stress of circumstances Koch bodies may appear in small numbers. It would appear that *Th. mutans* in (7) and (8) are identical.

In the family Theileridae there are therefore four named species that differ from one another in their virulence Doubt is expressed by du Toit (1930) whether the species of *Theileria* in cattle are good' pecies These species might all be regarded as varieties or strains of one species—*Th parva* Delpy (1949) has come to the conclusion that *Th dispar* is synonymous for *Th annulata* Recently Neitz (1955a) isolated a *Theileria* species from an African buffalo calf (*Syncerus caffer* Sparrman) in Zululand which is highly pathogenic for cattle (mortality 80 per cent) and distinct from *Th parva* He named this species *Theileria lawrencei* thus leaving four named species in the family Theileridae found in cattle

In sheep and goats two species of *Theileria* may be accepted provisionally *Theileria hirci* Dschunkowsky and Urodschevich 1924 is highly pathogenic, while *Th ovis* Rodhain 1916 is nonpathogenic Morphologically however these are indistinguishable The history of their nomenclature has been excellently reviewed by Thomson and Hall (1933)

The non pathogenic form [*Th ovis*] like *Theileria mutans* in cattle is associated with the presence of scanty Koch s blue bodies (Lestoquard 1929 Jansen and Neitz 1956) Rodhain (1916) described a parasite in sheep which did not seem to produce Koch s bodies and thus resembled *Theileria mutans* in that it was non virulent Rodhain considered this parasite in sheep as a new species and he named it *Theileria ovis* Yakimoff (1916) commented on Rodhain s paper and stated that at the third veterinary conference for Russia at Kharkov in 1913 he had already suggested the name *Theileria ovis* for this parasite In this statement quoted below Yakimoff (1916) gives no reference to a publication and we have been unable to trace in the literature any reference to a published account by Yakimoff in which the specific name suggested by him was mentioned

The name for the non pathogenic *Theileria* in sheep and goats would seem therefore to be *Theileria ovis* Rodhain 1916 Sergent Parrot and Hilbert (1922) named this parasite *Gonderia ovis* but Lestoquard (1929) having described Koch s blue bodies in such infections renamed the parasite *Theileria recondita* Wenyon (1926) in view of the fact that Koch s blue bodies had not been described in the non pathogenic form considered it justifiable to transfer this organism to the genus *Babesia* and in doing so suggested a new specific name *Babesia sergenti*

According to the evidence available the correct name for the non pathogenic *Theileria* of sheep is *Theileria ovis* Rodhain 1916 and *Babesia sergenti* Wenyon 1926 and *Theileria recondita* Lestoquard 1929 become synonyms

The pathogenic form in sheep with Koch s blue bodies was first described in Sudanese sheep by Mason 1915 and the following year in Egyptian sheep by Mason 1916 Mason placed this parasite in the genus *Theileria* but did not give a specific name In 1918 however du Toit in hi classification of the piropla ms gives this parasite the name *Theileria ovis* and attributes the name to Littlewood 1914 Donatien and Lestoquard (1930) without giving any references again quote *Theileria ovis* Littlewood 1914 as the correct name for the pathogenic form in sheep and goats The only justification for giving the name of Littlewood seems to be the fact that in his annual report he incor

porated the work of Mason This report was actually published in 1915 As far as can be ascertained there is no mention of species in Mason s account of this parasite either in 1915 or 1916 so that it would seem that neither Little wood nor Mason named this species

On the other hand du Toit (1918) records the name *Theileria ovis* which he attributes to Littlewood (1914) As the specific name *ovis* had however, been already used for the non pathogenic form *Theileria ovis* by Rodhain in 1916 it would seem that the correct name for the pathogenic form in sheep and goats is *Theileria hirci* Dschunkowsky and Urodschevich 1924 as correctly pointed out by Wenyon (1926) The name *Theileria ovis* wrongly attributed to Little wood in 1914 by du Toit must be abandoned

It is beyond the scope of this paper to consider the relatively large number of *Theileria* species in wild animals in detail Priestly (1915) described as *Th tachyglossi* a small parasite of the erythrocytes of *Tachyglossus aculeatus* an echidna of Australia The blood forms resembled those of *Th mutans* of cattle while in the organs and also in the blood structures resembling the schizonts of the same parasite were said to occur *Theileria* species have been described in the giraffe camel wart hog monkey polecat mouse and in several species of antelopes and deer In many of them no schizonts were found, and they were therefore originally included in the genus *Gonderia* In antelopes namely the eland (*Taurotragus* sp ) Lichtenheld (1911) Bright s gazelle (*Gazela* sp ) Montgomery (1924) topi (*Damaliscus* sp ) Hutchins (1924) hartebeest (*Sigmoceros* sp ) Ross (1924) and the bushbuck (*Tragelaphus* sp ) Theiler (1909) and Neitz (1931) Koch bodies as well as the erythrocytic stages of the parasites were found These observations prompted the investigators to include them in the genus *Theileria* From the description of *Cytauxzoon sylvicaprae* Neitz and Thomas 1948 however a parasite of the duiker (*Sylvicapra grimmia* L ) which multiplies by schizogony in the histiocytes and by fission in the erythrocytes it appears that at least some of the above mentioned *Theileria* species may in reality be *Cytauxzoon* species In order to avoid unnecessary confusion in the generic nomenclature only the species *Th tachyglossi* and *C sylvicaprae* of which the vertebrate life cycle is known will be included in the family Theileridae

The classification of the family Theileridae by du Toit (1918) which has been modified by Thomson and Hall (1933) Neitz and Thomas (1948) and Delpy (1949a) is as follows

Suborder **PIROPLASMIDEA** Wenyon 1926

Family **THEILERIDAE** du Toit 1918

Genus **THEILERIA** Bettencourt França and Borges 1907

Members of this genus are minute and rounded or rod shaped organisms Schizogony occurs in the cells of the lymphatic system Members of the family Ixodidae serve as vectors

*Th parva* (Theiler 1904)

*Th annulata* (Dschunkowsky and Luhs 1904)

Synonyms *Th dispar* Sergent *et al* 1924a b *Th turkestanica* Oboldueff and Galouzo 1928

*Th mutans* (Theiler 1906b)
Synonyms *Babesia mutans* Wenyon 1926 *Gonderia mutans* du Toit 1918

*Th hirci* Dschunkowsky and Urodschevich 1924
Synonym *Theileria ovis* du Toit 1918

*Th ovis* Rodhain 1916
Synonyms *Gonderia ovis* Lestoquard 1924 *Babesia sergenti* Wenyon 1926 *Theileria recondita* Lestoquard 1929

*Th tachyglossi* Priestly 1915

Genus CYTAUXZOON Neitz and Thomas 1948

*C sylvicaprae* Neitz and Thomas 1948

The nomenclature used in this classificatory list is based on the morphology and some biological characteristics presented by the Theilerias Consideration of these criteria shows that Theiler and Graf (1928) Sergent Donatien Parrot and Lestoquard (1929) Lestoquard (1929) and du Toit (1930) contend that schizogony in the lymphocytes and division of the erythrocytic forms into two or four daughter cells justified transferring *Gonderia mutans* and *Gonderia ovis* from the family Babesidae to the family Theileridae This mode of reproduction has also been observed in *Th annulata* (= *Th dispar*) by Sergent Donatien Parrot and Lestoquard (1945) in *Theileria laurencei* by Neitz (1955b) in *Th hirci* by Dschunkowsky and Luhs (1924) and Baumann (1939) and in *Th ovis* by Wenyon (1926) Sergent Parrot and Hilbert (1923) Lestoquard (1924 1926b) and Enigk (1953) In contradistinction to this mode of reproduction Wenyon (1926) states that *Th parva* multiplies only by schizogony in the lymphocytes and not by fission in the red blood cells Reichenow (1940) also failed to demonstrate any divisional forms in the erythrocytes of cattle affected with East Coast fever In this connection it should be mentioned that Neitz and Jansen (1956) have had an opportunity of studying the life cycle of pure infections of *Th annulata Th mutans* and *Th ovis* in the vertebrate hosts The infectious agents were transmitted by means of infected ticks to animals known to be free from blood parasites Splenectomy of naturally recovered animals was followed by a reappearance of the erythrocytic stages of these parasites within a period of three weeks after the operation Divisional forms could readily be demonstrated in the peripheral circulation thus confirming the observations of previous workers In the case of *Th laurencei* Neitz (1955b) observed that the parasites persisted in the peripheral circulation of a buffalo for a period of four months (end point not determined) and that nymphal ticks (*Rhipicephalus appendiculatus*) that fed on this animal transmitted the infection in the ensuing stage *Theileria laurencei* multiplies by schizogony in the lymphocytes and by binary fission in the erythrocytes giving rise to four daughter individuals In contradistinction to this manifestation du Toit (1928) and Neitz (1948) established that splenectomy did not interrupt the immunity in East Coast fever and that the erythrocytic stages of *Th parva* failed to appear for periods of up to eight weeks after the operation

The difference between the behavior of the erythrocytic stages of *Th parva*

porated the work of Mason This report was actually published in 1915 As far as can be ascertained there is no mention of species in Mason s account of this parasite either in 1915 or 1916 so that it would seem that neither Littlewood nor Mason named this species

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Family **THEILERIDAE** du Toit 1918

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*Th annulata* (Dschunkowsky and Luhs 1904)

Synonyms *Th dispar* Sergent *et al* 1924a b *Th turkestanica* Oboldueff and Galouzo 1928

criteria for what is believed to be a more satisfactory basis for their classification Neitz and Jansen (1956) submitted in summary form the revision of the classification For the sake of completeness the generic and specific names as well as the generic and specific synonymy will be included It is self evident that a large number of *Theileria* species or *Gonderia* species described from wild animals cannot be incorporated in this scheme In order to avoid further confusion it is suggested that they be referred to by the generic name already assigned to them and that their inclusion into this system be undertaken when all the phases of the life cycle in the vertebrate host have been established

The revised classification of the family Theileridae is as follows

Suborder **LEUCOSPORIDEA** Neitz and Jansen 1956

In this suborder are included certain parasites that inhabit either lymphocytes or histiocytes and erythrocytes but do not form pigment (haemozoin) characteristic of members of the suborder Haemosporidiidea Danilewsky They multiply by schizogony and finally invade the erythrocytes within which they occur as round ovoid rodlike or irregular forms These parasites as far as is known are transmitted by ticks of the family Ixodidae Murray 1877 There are two families in this suborder the Theileridae and Gonderidae

Family **THEILERIDAE** du Toit 1918

Parasites that multiply by schizogony in the lymphocytes and finally invade the erythrocytes The forms in the red blood corpuscles do not reproduce and are possibly gametocytes or gametes The family is represented by a single genus *Theileria*

Genus **THEILERIA** Bettencourt França and Borges 1907

*Theileria parva* (Theiler 1904)

Synonyms *Piroplasma kochi* Stephens and Christophers 1903 *Piroplasma parvum* Theiler 1904 *Theileria kochi* Stephens and Christophers 1903

Family **GONDERIDAE** Neitz and Jansen 1956

Parasites that multiply by schizogony in either the lymphocytes (*Gonderia* sp ) or histiocytes (*Cytauxzoon* sp ) and finally invade the red blood corpuscles The forms in the erythrocytes reproduce by division into two or four daughter individuals the latter giving rise to the characteristic cross forms The final stage of the parasites is possibly a gametocyte or a gamete This family is represented by two genera *Gonderia* and *Cytauxzoon*

Genus **GONDERIA** (du Toit 1918)

Members of this genus *multiply by schizogony in the lymphocytes and by* fission in the erythrocytes

*Gonderia annulata* (Dschunkowsky and Luhs 1904)

Synonyms *Piroplasma annulatum* Dschunkowsky and Luhs 1904 *Theileria annulata* (Dschunkowsky and Luhs 1904) *Theileria dispar* Sergent *et al* 1924a b *Theileria turkestanica* Oboldeuff and Galouzo 1928

and that of the remaining *Theileria* species and the *Cytauxzoon* species (*vide supra*) is regarded as significant and as a sound reason for revising the classification of these microorganisms, particularly if one considers the basis on which the *Plasmodia* species (schizogony within the endothelial cells lining the blood capillaries or hepatic cells as well as within the erythrocytes) are differentiated from the *Haemoproteus* species (schizogony only within the endothelial cells lining the blood capillaries) In accepting the behavior of the erythrocytic stages of the *Theileria* species as an additional classificatory basis it is proposed to retain the generic and specific name of *Theileria parva* and to transfer the remaining *Theileria* species to a separate genus In order to avoid unnecessary confusion in the nomenclature, it is suggested that the generic name *Gonderia* (du Toit 1918) be reinstated and that for the purpose of this classification it be redefined as indicated below in the revised classificatory list

Reasons have been given why the *Theileria* species should be placed into three separate genera namely *Theileria* Bettencourt, França and Borges 1907 *Gonderia* (du Toit 1918) and *Cytauxzoon* Neitz and Thomas 1948 The question now arises as to whether these three genera should be retained in the family Theileridae Consideration of the criteria used for distinguishing between the family Plasmodiidae Mesnil 1903 and the family Haemoproteidae Doflein 1916 (*vide supra*) suggests that in principle they are equally applicable in the case of the genera *Theileria Gonderia* and *Cytauxzoon* It is therefore proposed that *Th parva* (Theiler 1904) be retained in the family Theileridae and that *Gonderia (Theileria) annulata* (Dschunkowsky and Luhs 1904) *Gonderia (Theileria) mutans* (Theiler 1906) *Gonderia (Theileria) lawrencei* (Neitz 1955a) *Gonderia (Theileria) hirci* (Dschunkowsky and Urodschevich 1924) *Gonderia (Theileria) ovis* (Rodhain 1916) *Gonderia (Theileria) tachyglossi* (Priestly 1915) and *Cytauxzoon sylvicaprae* (Neitz and Thomas 1948) be included in a separate family for which the name Gonderidae is proposed The definitions for the families Theileridae and Gonderidae are given below in the classifacatory list

The last question of systematic importance that arises out of this discussion is whether or not the two families Theileridae and Gonderidae should be retained in the suborder Piroplasmidea Wenyon 1926 The difference between the developmental cycle of the family Babesidae (absence of a schizogonous cycle) and that of the families Theileridae and Gonderidae (presence of the schizogonous cycle in the leukocytes) is so great that there is no real justification for retaining the latter two families in the suborder Piroplasmidea It is therefore suggested that the families Theileridae and Gonderidae be placed into a new suborder for which the name Leucosporidea is proposed In doing so Neitz and Jansen (1956) accept the view of Gonder (1910 1911) Cowdry and Danks (1933) and Sergent Donatien Parrot and Lestoquard (1945) that *Theileria parva* and *Gonderia (Theileria) annulata* (= *Theileria dispar*) multiply by schizogony and sporogony a process of reproduction characteristic of the order Coccidia Leuckart and of the class Sporozoa Leuckart The definition for the suborder Leucosporidea is given below in the classificatory list

Having surveyed in detail the complicated history of the nomenclature of the protozoa commonly referred to as *Theileria* parasites and having indicated the

zones also have their share The piroplasms are exclusively transmitted by members of the family Ixodidae Murray

The various ticks concerned in the transmission are listed in a series of tables based on the method indicated by du Toit (1918) If the present tables are compared with the records of Knuth and du Toit (1921) and Neitz and du Toit (1938) it will be seen that a large number of new vectors have been added At this stage the writer wishes to express his sincere thanks to Gertrud Theiler of the Onderstepoort Veterinary Institute for her invaluable assistance in determining the correct nomenclature of a large number of ticks particularly the *Hyalomma* species and *Boophilus* species It is hoped that these lists will be of value to veterinarians engaged in the control of tick borne diseases and to zoologists occupied in determining the species or their distribution

## Transmission of the Members of the Order Piroplasmidea

### *Biological Transmission*

*Babesia* species of cattle

*Babesia bigemina* *B bigemina* was the first protozoon parasite shown to be transmitted by an arthropod It will be seen from TABLE 1 that this microorganism is widely distributed in the world Five tick species of the genus *Boophilus* three species of *Rhipicephalus* and one species of *Haemaphysalis* are known to be vectors In case of the one host ticks *Boophilus* species the infection passes through the egg stage and is given off by the larvae in the next generation Theiler (1909c) was able to show that *B decoloratus* retained the infection to the second generation In the *Rhipicephalus* species and *Haemaphysalis* species stage to stage transmission within the same generation as well as hereditary transmission has been recorded

The first records of the life cycle of *B bigemina* in the tick are those of Koch (1906) and Christophers (1907b) Dennis (1932) studied its development in *B annulatus* but according to Reichenow (1935) some of the stages described are not those of *B bigemina* but those of symbionts Regendanz (1936) conducted his observations in *B microplus* The process of development very closely resembles that of *B canis* in *D reticulatus* He failed to find however any parasites in the salivary glands

It is interesting and of great significance to note that the systematic destruction of *B annulatus* has almost eliminated *B bigemina* infection from the United States of America

*Babesia bovis* So far two species of *Ixodes* have been established as vectors of *B bovis* in Europe Both ticks transmit the disease hereditarily as shown in TABLE 2 Stage to stage transmission within the same generation has been demonstrated in *I ricinus*

*Babesia argentina* Two species of *Boophilus* serve as vectors of *B argentina* The experimental observations are listed in TABLE 3

*Babesia berbera* The two vectors of *B berbera* in North Africa are mentioned in TABLE 4 Infected larvae and nymphae of the two-host tick *Rh bursa* transmit the infection in the adult stage while *B calcaratus* transmits the disease hereditarily

*Gonderia mutans* (Theiler 1906b)
Synonyms *Piroplasma mutans* (Theiler, 1906) *Theileria mutans* (Theiler 1906)

*Gonderia lawrencei* (Neitz 1955a)
Synonym *Theileria lawrencei* Neitz 1955a

*Gonderia hirci* (Dschunkowsky and Urodschevich 1924)
Synonyms *Theileria ovis* du Toit 1918 *Theileria hirci* Dschunkowsky and Urodschevich 1924

*Gonderia ovis* (Rodhain 1916)
Synonyms *Theileria ovis* Rodhain 1916 *Babesia sergenti* Wenyon 1926 *Gonderia ovis* Lestoquard 1924 *Theileria recondita* Lestoquard 1929

*Gonderia tachyglossi* (Priestly 1915)
Synonym *Theileria tachyglossi* Priestly, 1915

### Genus CYTAUXZOON Neitz and Thomas 1948

Members of this genus multiply by schizogony in cells of the histiocytic series and by fission in the erythrocytes

*Cytauxzoon sylvicaprae* Neitz and Thomas 1948

### Conclusions Regarding the Classification

The morphological similarity between the members of the genera *Theileria* and *Gonderia* in domestic animals makes their differentiation extremely difficult In making a differential diagnosis veterinarians and zoologists often are compelled to take the pathogenicity and the epizootiology into consideration In doubtful cases it may be necessary even to resort to *in vivo* cross immunity tests before a final identification can be made From this it becomes evident that a great deal of work will have to be done before the intermediate stages of the *Theileria* species referred to by du Toit (1930) can be identified From the observations on the artificial transmission of East Coast fever (Theiler 1912 Theiler and du Toit 1929) and of Tzaneen disease (*Gonderia mutans* infection) (de Kock van Heerden du Toit and Neitz 1937) it becomes apparent that the infectious agents cannot be maintained by serial passages as has been possible in the case of *Gonderia annulata* (Sergent Donatien Parrot and Lestoquard 1945) The opinion is expressed that the undetermined species referred to by du Toit (1930) may behave in a similar way For systematic studies therefore it will be necessary to determine the vectors of these parasites From this it is obvious that many years will elapse before the classification of this group of microorganisms can be placed on a permanent basis

### Transmission of the Piroplasms

The relationship between ticks and redwater in cattle was suspected by stock owners in America as early as 1869 but it was only in 1893 that Smith and Kilborne demonstrated that *Boophilus annulatus* was the vector of the disease Although tick borne diseases are more prevalent in the tropics the temperate

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Rhipicephalus appendiculatus* Neum | South Africa | 3 | | X— | —)<br>X— | — | —) | | | Theiler (1909a) |
| *Rhipicephalus bursa* Canestrini and Fanzago | North Africa | 2 | X— | —X— | —)<br>X— | — | —)— | —)— | —) | Sergent *et al* (1931)<br>Sergent Donatien Parrot and Lestoquard (1945) |
| *Rhipicephalus evertsi* Neum | South Africa | 2 | X— | —X— | —)<br>X— | — | —) | | | Theiler (1909a) |

Note X denotes the stage in which the infection was acquired ) the stage in which the infection was transmitted

TABLE 2

THE BIOLOGICAL TRANSMISSION OF BABESIA BOVIS BY MEANS OF TICKS

| Vector | Country | No of hosts | Larva | Nymph | Imago | Egg | Larva | Nymph | Imago | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| *Ixodes persulcatus* Schulze | Russia | 3 | | | X— | — | —)— | —) | | Gouseff Rastegaieff and Sousko (1936) |
| *Ixodes ricinus* (Linn ) | Europe | 3 | | | X— | — | —)<br>X— | —)<br>X— | —) | Kossel Schütz Weber and Miessner (1903) |

TABLE 3

THE BIOLOGICAL TRANSMISSION OF BABESIA ARGENTINA BY MEANS OF TICKS

| Vector | Country | No of hosts | Larva | Nymph | Imago | Egg | Larva | Nymph | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Boophilus microplus* (Canestrini) | Argentine | 1 | X— | —X— | —X— | — | —) | | | Lignières (1903) |
| *Boophilus australis* Fuller [referred to as *Boophilus microplus* (Canestrini) in the Australian literature] | Australia | 1 | X— | —X— | —X— | — | —) | | | Dodd (1910)<br>Legg (1935) |

TABLE I
THE BIOLOGICAL TRANSMISSION OF BABESIA BIGEMINA BY MEANS OF TICKS

| Vectors | Country | No. of hosts | Larva | Nympha | Imago | Egg | Larva | Nympha | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Boophilus annulatus* (Say) | North America | 1 | X— | —X— | —X— | —— | —) | | | Smith and Kilborne (1893) |
| *Boophilus australis* Fuller [referred to as *Boophilus microplus* (Canestrini) in Australian literature] | Australia | 1 | X— | —X— | —X— | —— | —) | | | Hunt and Collins (1896)<br>Pound and Hunt (1895) |
| *Boophilus australis* Fuller [may actually be *Boophilus microplus* (Canestrini)] | Panama | 1 | X— | —X— | —X— | —— | —) | | | Clark (1918)<br>Clark and Zetek (1925) |
| *Boophilus calcaratus* Birula [referred to as *Boophilus annulatus calcaratus* (Birula)] | North Africa | 1 | X— | —X— | —X— | —— | —) | | | Brumpt (1920)<br>Sergent, Donatien, Parrot and Lestoquard (1945) |
| *Boophilus decoloratus* (Koch) | South Africa | 1 | X—<br>X—<br>) | —X—<br>—X— | —X—<br>—X— | ——<br>—— | —)<br>—)— | <br>—— | <br>— | Koch (1898)<br>Laveran and Vallée (1905)<br>Theiler (1904, 1909c) |
| *Boophilus microplus* (Canestrini) | South America | 1 | X— | —X— | — X | —— | —) | | | Lignières (1900, 1901)<br>Ziemann (1902)<br>Brumpt (1920)<br>Rosenbusch and Gonzales (1924)<br>Regendanz (1936) |
| *Haemaphysalis punctata* (Canestrini and Fanzago) [referred to as *Haemaphysalis cinnabarina punctata* Canestrini and Fanzago] | Europe | 3 | | X— | —)<br>X— | <br>—— | <br>—— | <br>—— | <br>—) | Knuth (1915a, b)<br>Zeller and Helm (1923) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Rhipicephalus appendiculatus* Neum | South Africa | 3 | | X— | —) X— | — — | —) | | | Theiler (1909a) |
| *Rhipicephalus bursa* Canestrini and Fanzago | North Africa | 2 | X— | X— | —) X— | —— | —) | —) | —) | Sergent *et al* (1931) Sergent Donatien Parrot and Lestoquard (1945) |
| *Rhipicephalus evertsi* Neum | South Africa | 2 | X | X— | —) X— | —— | —) | | | Theiler (1909a) |

Note X denotes the stage in which the infection is acquired and ) the stage in which the infection is transmitted

TABLE 2

THE BIOLOGICAL TRANSMISSION OF BABESIA BOVIS BY MEANS OF TICKS

| Vector | Country | No. of hosts | Larva | Nymph | Imago | Egg | Larva | Nymph | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Ixodes persulcatus* Schultze | Russia | 3 | | | X— | —— | —) | —) | | Cousseff Rastegaeff and Sousk (1936) |
| *Ixodes ricinus* (Linn.) | Europe | 3 | | | X— | —— | —) X— | —) X— | —) | Kossel Schütz Weber and Miessner (1903) |

TABLE 3

THE BIOLOGICAL TRANSMISSION OF BABESIA ARGENTINA BY MEANS OF TICKS

| Vector | Country | No. of hosts | Larva | Nymph | Imago | Egg | Larva | Nymph | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Boophilus microplus* (Canestrini) | Argentine | 1 | X— | —X— | —X— | —— | —) | | | Lignières (1903) |
| *Boophilus australis* Fuller [referred to as *Boophilus microplus* (Canestrini) in the Australian literature] | Australia | 1 | X— | —X— | —X— | —— | —) | | | Dodd (1910) Legg (1935) |

TABLE 4
THE BIOLOGICAL TRANSMISSION OF BABESIA BERBERA BY MEANS OF TICKS

| Vector | Country | No. of hosts | Larva | Nympha | Imago | Egg | Larva | Nympha | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Boophilus (annulatus) calcaratus* Birula | North Africa | 1 | X— | —X— | —X— | ——— | ——) | | | Sergent, Donatien, Parrot and Lestoquard (1945) |
| *Rhipicephalus bursa* Canestrini and Fanzago | North Africa | 2 | X— | —X— | ——) | | | | | Sergent, Donatien, Parrot and Lestoquard (1945) |

TABLE 5
THE BIOLOGICAL TRANSMISSION OF BABESIA MOTASI BY MEANS OF TICKS

| Vector | Country | No. of hosts | Larva | Nympha | Imago | Egg | Larva | Nympha | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Dermacentor silvarum* Oken | Southern Russia | 3 | ? | | | | | | | Rastegaieff (1936) |
| *Haemaphysalis punctata* Canestrini and Fanzago | Sardinia | 3 | ? | | | | | | | Pegreffi and Mura (1948) |
| *Rhipicephalus bursa* Canestrini and Fanzago | Roumania | 2 | <br>X— | <br>—X— | X—<br>——) | ——— | ——— | ——— | ——) | Motas (1904)<br>Rastegaieff (1933, 1936) |

TABLE 6
THE BIOLOGICAL TRANSMISSION OF BABESIA OVIS BY MEANS OF TICKS

| Vector | Country | No. of hosts | Larva | Nympha | Imago | Egg | Larva | Nympha | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Rhipicephalus bursa* Canestrini and Fanzago | Russia | 2 | X—<br><br><br>—— | —X—<br><br>——<br>——— | ——)<br>X—<br>——<br>——) | <br>———<br>——— | <br>———<br>——— | <br>———<br>——— | <br>——<br>—— | Rastegaieff (1933, 1936)<br>Markov and Kurchatov (1940a) |

*Babesia major* The vectors of *B major* have apparently not yet been established

*Basbesia* species of sheep and goats

*Babesia motasi* The vectors of *B motasi* are listed in TABLE 5 Stage to-stage transmission within the same generation and hereditary transmission have been demonstrated in *Rh bursa* The mode of transmission in the case of *D silvarum* and *H punctata* has not yet been worked out in detail The investigators merely state that they are vectors

*Babesia ovis* Only *Rh bursa* has so far been established as a vector (TABLE 6) Stage to stage as well as hereditary transmission has been established Markov and Kurchatov (1940a) allowed the descendants of a female (*Rh bursa*) taken from a ram affected with *B ovis* to feed on a horse The following generation of these ticks caused sheep to sicken with babesiosis The next two generations of these ticks obtained from females that had fed on horses were fed on a rabbit and on a calf The following generation was placed on rams All animals developed babesiosis and one died

*Babesia foliata* and *Babesia taylori* Both these *Babesia* species have been described in India Their vectors have apparently not yet been identified

*Babesia* species of solipeds

Not only has Enigk (1943 1944a 1951) given an excellent review on the various vectors of equine babesiosis but he has identified eight new vectors in Europe The distribution and biological habits of these ticks are described in detail Observations are recorded on the survival of certain ticks as well as as the parasites harbored by them at temperatures below 0 C The articles should be consulted by those interested in the biological habits of European ticks

*Babesia caballi* The vectors of *B caballi* are recorded in TABLE 7 Three species of the genus *Dermacentor* four species of *Hyalomma* and two species of *Rhipicephalus* are known to be vectors Stage to-stage transmission has been demonstrated in all of them except in *H anatolicum* and *H volgense* and hereditary transmission in all but *D pictus H anatolicum* and *Rh bursa* Enigk (1944a) established that *Rh sanguineus* can retain *B caballi* for four generations

*Babesia equi* The vectors of *B equi* are recorded in TABLE 8 Two pecies of the genus *Dermacentor* four species of the *Hyalomma* and three species of *Rhipicephalus* are known to be vectors In contradistinction to *B caballi B equi* is only transmitted hereditarily by one pecies of tick namely *H anatolicum* In the remaining vectors stage to stage transmission has been established

*Babesia* species of swine

*Babesia trautmanni* According to Kurchatov and Markov (1940) *Rhipicephalus turanicus* Pomerantzer and Matitoshvily is a vector of *B trautmanni* Successful infection was effected with the progeny of two engorged females The larvae and nymphae were fed on rabbits and the adults conveyed the disease to swine Circumstantial evidence suggests that *B decoloratus* is a vector in Tanganyika (Trautmann 1914 Knuth and du Toit 1921) and that

TABLE 7
THE BIOLOGICAL TRANSMISSION OF BABESIA CABALLI BY MEANS OF TICKS

| Vector | Country | No. of hosts | Larva | Nympha | Imago | Egg | Larva | Nympha | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Dermacentor marginatus* Sulzer (= *Dermacentor reticulatus* Fabricius) | Southern Russia | 3 | | | × — | — | — | — | — ) | Marzinowsky and Bielitzer (1909) |
| | Germany | | × — | — ) | | | | | | du Toit (1919) |
| | European Russia | | | × — | — ) | | | | | Enigk (1944a) |
| | | | | × — | — ) | | | | | |
| *Dermacentor pictus* Herm. | European Russia | 3 | | × — | — ) | | | | | Enigk (1944a) |
| *Dermacentor silvarum* Olen. | European Russia | 3 | × — | — | — | — | — | — | — ) | Markov, Bogoroditsky and Salyaev (1935) |
| | European Russia | | × — | — × — | — × — | — | — | — | — ) | Dzasokhov and Cyrgan (1939) |
| | Ukraine | | | | × — | — | — | — ) | | Lohorilya (1937) |
| | | | | | × — | — | — | — | — ) | |
| *Hyalomma anatolicum* Koch (= *Hyalomma excavatum* Koch according to Delpy 1949a and Feldman-Muhsam 1954) | Greece | 3 | ? | ? | — ) | | | | | Enigk (1943) |
| *Hyalomma dromedarii* Koch | North Africa | 3 | | | × — | — | — | — | — ) | Enigk (1943) |
| *Hyalomma marginatum* Koch (= *Hyalomma detritum* Schulze according to Delpy 1949a) (= *Hyalomma marginatum* according to Feldman-Muhsam 1954) | Greece | 3 | × — | — | — ) | | | | | Enigk (1943) |
| | | | | | × — | — | — | — | — ) | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Hy l mia lgense* S hulz and Schlotke | Ukraine | 1 or 2 | | | X— | — | — | — | —) | Engh (1944a) |
| *Rhip cephalu bursa* Cane trini and Fanzag | Bulgaria | 2 | X— | —X— | —) | | | | | Engh (1943) |
| *Rhipicephalus sanguineus* (Latreill ) | Greece | 3 | X— | —) | X— | — | — | — | —) | Engh (1943 1944a) |

TABLE 8
THE BIOLOGICAL TRANSMISSION OF BABESIA EQUI BY MEANS OF TICKS

| Vector | Country | No. of hosts | Larva | Nymph | Imago | Egg | Larva | Nympha | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Dermacentor marginatus* Sulzer = (*Dermacentor reticulatus* Fabricius) | European Russia | 3 | | X— | —) | | | | | Enigk (1944a) |
| *Dermacentor pictus* Herm | European Russia | 3 | | X— | —) | | | | | Enigk (1943) |
| *Hyalomma anatolicum* Koch = (*Hyalomma excavatum* Koch according to Delpy 1949b and Feldman Muhsam 1954) | Greece | 3 | ? | ? | —)<br>X— | — | — | — | —) | Enigk (1943) |
| *Hyalomma dromedarii* Koch | North Africa | 3 | | X— | —) | | | | | Enigk (1943) |
| *Hyalomma marginatum* Koch<br>= (*Hyalomma detritum* according to Delpy 1949b)<br>= *Hyalomma marginatum* according to Feldman Muhsam 1954 | North Caucasia<br>Ukraine<br>Central Asia<br>Greece | 3 | ?<br>?<br>X— | ?<br>?<br>— | —)<br><br>—) | | | | | Nikolsky (1933)<br>Agrinsky (1937)<br>Enigk (1944) |
| *Hyalomma* ... Schulz and Schlottke 1929 = (*Hyalomma detritum* Schulze according to Delpy 1949) | Caucasia | 1 or 2? | X— | — | —) | | | | | Enigk (1944a) |
| *Rhipicephalus bursa* Canestrini and Fanzago | Russia | 2 | X— | —X— | —) | | | | | Markov and Kurchatov (1940b)<br>Markov Kurchatov and Dzasochov (1940) |
| *Rhipicephalus evertsi* Neum | South Africa | 2 | X— | —X— | —) | | | | | Theiler (1906a) |
| *Rhipicephalus sanguineus* (Latreille) | Central Asia<br>North Africa | 3 | ?<br>X— | ?<br>—)<br>X— | <br><br>—) | | | | | Agrinsky (1937)<br>Enigk (1943) |

*Rh sanguineus* (Cerruti 1939 Nardi and Lo Muzio 1952) *Hyalomma aegyptium* Linn and *Dermacentor reticulatus* (Cerruti 1939) transmit the infection in Europe Attempts by Kurchatov and Markov (1940) to transmit *B trautmanni* with *Hyalomma marginatum* *Rhipicephalus sanguineus* *Rh rossicus* and *Dermacentor silvarum* gave negative results (*Rh turanicus* Pomerantzev and Matikashvily according to Zumpt (1948) is a synonym for *Rh sanguineus* (Latreille) *Rh rossicus* Yakimoff and Yakimoff according to Zumpt is a subspecies of *Rh sanguineus* but according to Feldman Mühsam (1952) a distinct species *H aegyptium* Linn is an ectoparasite of reptiles and it is possible that the species was not correctly identified )

*Babesia perroncitoi* The vectors of *B perroncitoi* have not yet been established experimentally Cerruti (1939) suggests that *Rh sanguineus* *D reticulatus* and *Hyalomma aegyptium* may be vectors in Italy

*Babesia* species of the canine family

*Babesia canis* The six vectors of *B canis* are listed in the appended TABLE 9 Hereditary transmission occurs in all of them except in *D pictus* Stage-to-stage transmission within the same generation has been observed in *D pictus* *H leachi* and *Rh sanguineus*

The chief transmitter the cosmopolitan dog tick *Rh sanguineus* has been recorded from Africa North and South America Asia and Europe Although this tick occurs in Australia canine babesiosis has not yet been encountered on that continent (Newton 1951) In Europe it appears that *D marginatus* is the chief vector *D pictus* is responsible for canine winter piroplasmosis (Enigk 1944b) *H marginatum* is not an important vector as the adult stage is seldom seen on dogs

Although Brumpt and Larrouse (1922) proved that the American Rocky Mountain spotted fever tick (*D venustus*) is a vector of *B canis* no mention is made in the literature about its role as a transmitter in the United States of America Morgan and Hawkin (1948) believe that the American dog tick *D variabilis* (Say) is also a vector in the United States

Brumpt observed that *B canis* can be retained in the tick *Rh sanguineus* for five generations

Studies on the life cycle of *B canis* in ticks have been conducted by Christophers (1907a b) Regendanz and Muniz (1936) and Shortt (1936) in *Rh sanguineus* and by Regendanz and Reichenow (1933) and Brumpt (1937) in *D marginatus* Brumpt (1937) failed to demonstrate any developmental stages of *B canis* in *H leachi* Consideration of the life cycle of *B canis* in the vectors caused Reichenow (1935 1953) to conclude that sporogony does not occur Multiplication in the vertebrate and invertebrate hosts follows simple binary fission

*Babesia gibsoni* Two vectors have been established in the case of *B gibsoni* Stage to stage transmission occurs in *H bispinosa* and *Rh sanguineus* In the former tick hereditary transmission has also been observed Details of the observations are given in TABLE 10

*Babesia felis* of the feline family

Very little is known about the natural transmission of *B felis* Davis

TABLE 8
THE BIOLOGICAL TRANSMISSION OF BABESIA EQUI BY MEANS OF TICKS

| Vector | Country | No. of hosts | Larva | Nymph | Imago | Egg | Larva | Nymph | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Dermacentor marginatus* Sulzer = (*Dermacentor reticulatus* Fabricius) | European Russia | 3 | | X— | —) | | | | | Enigk (1944a) |
| *Dermacentor pictus* Herm | European Russia | 3 | | X— | —) | | | | | Enigk (1943) |
| *Hyalomma anatolicum* Koch = (*Hyalomma excavatum* Koch according to Delpy 1949b and Feldman Muhsam 1954) | Greece | 3 | ? | ? | —) X— | — | — | — | —) | Enigk (1943) |
| *Hyalomma dromedarii* Koch | North Africa | 3 | | X— | —) | | | | | Enigk (1943) |
| *Hyalomma marginatum* Koch = (*Hyalomma detritum* according to Delpy 1949b) = *Hyalomma marginatum* according to Feldman Muhsam 1954 | North Caucasia Ukraine Central Asia Greece | 3 | ? ? X— | ? ? —— | —) —) | | | | | Nikolsky (1933) Agrinsky (1937) Enigk (1944) |
| *Hyalomma ... dense* Schulze and Schlottke 1929 = (*Hyalomma detritum* Schulze according to Delpy 1949) | Caucasia | 1 or 2? | X— | —— | —) | | | | | Enigk (1944a) |
| *Rhipicephalus bursa* Canestrini and Fanzago | Russia | 2 | X— | —X— | —) | | | | | Markov and Kurchatov (1940b) Markov Kurchatov and Dsasochov (1940) |
| *Rhipicephalus evertsi* Neum | South Africa | 2 | X— | —X— | —) | | | | | Theiler (1906a) |
| *Rhipicephalus sanguineus* (Latreille) | Central Asia North Africa | 3 | ? X— | ? —) X— | —) | | | | | Agrinsky (1937) Enigk (1943) |

*Rh sanguineus* (Cerruti 1939 Nardi and Lo Muzio 1952) *Hyalomma aegyptium* Linn and *Dermacentor reticulatus* (Cerruti, 1939) transmit the infection in Europe Attempts by Kurchatov and Markov (1940) to transmit *B trautmanni* with *Hyalomma marginatum Rhipicephalus sanguineus Rh rossicus* and *Dermacentor silvarum* gave negative results (*Rh turanicus* Pomerantzev and Matitoshvily according to Zumpt (1948) is a synonym for *Rh sanguineus* (Latreille) *Rh rossicus* Yakimoff and Yakimoff according to Zumpt is a subspecies of *Rh sanguineus* but according to Feldman Muhsam (1952) a distinct species *H aegyptium* Linn is an ectoparasite of reptiles and it is possible that the species was not correctly identified )

*Babesia perroncitoi* The vectors of *B perroncitoi* have not yet been established experimentally Cerruti (1939) suggests that *Rh sanguineus D reticulatus* and *Hyalomma aegyptium* may be vectors in Italy

*Babesia* species of the canine family

*Babesia canis* The six vectors of *B canis* are listed in the appended TABLE 9 Hereditary transmission occurs in all of them except in *D pictus* Stage to stage transmission within the same generation has been observed in *D pictus H leachi* and *Rh sanguineus*

The chief transmitter the cosmopolitan dog tick *Rh sanguineus* has been recorded from Africa North and South America Asia and Europe Although this tick occurs in Australia canine babesiosis has not yet been encountered on that continent (Newton 1951) In Europe it appears that *D marginatus* is the chief vector *D pictus* is responsible for canine winter piroplasmosis (Enigk 1944b) *H marginatum* is not an important vector as the adult stage is seldom seen on dogs

Although Brumpt and Larrouse (1922) proved that the American Rocky Mountain spotted fever tick (*D venustus*) is a vector of *B canis* no mention is made in the literature about its role as a transmitter in the United States of America Morgan and Hawkin (1948) believe that the American dog tick *D variabilis* (Say) is also a vector in the United States

Brumpt observed that *B canis* can be retained in the tick *Rh sanguineus* for five generations

Studies on the life cycle of *B canis* in ticks have been conducted by Christophers (1907a b) Regendanz and Muniz (1936) and Shortt (1936) in *Rh sanguineus* and by Regendanz and Reichenow (1933) and Brumpt (1937) in *D marginatus* Brumpt (1937) failed to demonstrate any developmental stages of *B canis* in *H leachi* Consideration of the life cycle of *B canis* in the vectors caused Reichenow (1935 1953) to conclude that sporogony does not occur Multiplication in the vertebrate and invertebrate hosts follows simple binary fission

*Babesia gibsoni* Two vectors have been established in the case of *B gibsoni* Stage to stage transmission occurs in *H bispinosa* and *Rh sanguineus* In the former tick hereditary transmission has also been observed Details of the observations are given in TABLE 10

*Babesia felis* of the feline family

Very little is known about the natural transmission of *B felis* Davis

**Table 8**
**The Biological Transmission of Babesia Equi by Means of Ticks**

| Vector | Country | No. of hosts | L | Nymph | Imago | Egg | L | Nymph | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Dermacentor marginatus* Sulzer = (*Dermacentor reticulatus* Fabricius) | European Russia | 3 | | ×— | —) | | | | | Enigk (1944a) |
| *Dermacentor pictus* Herm | European Russia | 3 | | ×— | —) | | | | | Enigk (1943) |
| *Hyalomma anatolicum* Koch = (*Hyalomma excavatum* Koch according to Delpy 1949b and Feldman Muhsam 1954) | Greece | 3 | ? | ? | —)<br>×— | — | — | — | —) | Enigk (1943) |
| *Hyalomma dromedarii* Koch | North Africa | 3 | | ×— | —) | | | | | Enigk (1943) |
| *Hyalomma marginatum* Koch = (*Hyalomma detritum* according to Delpy 1949b) = *Hyalomma marginatum* according to Feldman Muhsam 1954 | North Caucasia<br>Ukraine<br>Central Asia<br>Greece | 3 | ?<br>?<br>×— | ?<br>?<br>— | —)<br><br>—) | | | | | Nikolsky (1933)<br>Agrinsky (1937)<br>Enigk (1944) |
| *Hyalomma uralense* Schulze and Schlottke 1929 = (*Hyalomma detritum* Schulze according to Delpy 1949) | Caucasia | 1 or 2? | ×— | — | —) | | | | | Enigk (1944a) |
| *Rhipicephalus bursa* Canestrini and Fanzago | Russia | 2 | ×— | —×— | —) | | | | | Markov and Kurchatov (1940b)<br>Markov Kurchatov and Dyasochov (1940) |
| *Rhipicephalus evertsi* Neum | South Africa | 2 | ×— | —×— | —) | | | | | Theiler (1906a) |
| *Rhipicephalus sanguineus* (Latreille) | Central Asia<br>North Africa | 3 | ?<br>×— | ?<br>—)<br>×— | <br><br>—) | | | | | Agrinsky (1937)<br>Enigk (1943) |

TABLE 10

THE BIOLOGICAL TRANSMISSION OF BABESIA GIBSONI BY MEANS OF TICKS

| Vector | Country | No. of hosts | Larva | Nymph | Imago | Egg | Larva | Nymph | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Haemaphysalis bispinosa* Neum | India | 3 | <br><br><br>X—<br> | X—<br><br><br>—)<br>X— | —)<br>X—<br>X—<br><br>—) | <br>——<br>——<br><br> | <br>—)<br>—)<br><br> | <br>—)<br><br><br> | | Swaminath and Shortt (1937)<br>Shortt (1938) |
| *Rhipicephalus sanguineus* (Latreille) | India | 3 | X—<br> | —)<br>X— | <br>—) | | | | | Sen (1933)<br>Datta (1940) |

TABLE 9
THE BIOLOGICAL TRANSMISSION OF BABESIA CANIS BY MEANS OF TICKS

| Vectors | Country | No. of hosts | Larva | Nympha | Imago | Egg | Larva | Nymph | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Dermacentor marginatus* Sulzer = (*Dermacentor reticulatus* Fabricius) | Europe | 3 | | | X— | —— | —— | —— | —) | Brumpt (1919) |
| | | | | | X— | —— | —— | —— | —) | Belitzer and Markoff (1930) |
| | | | | | X— | —— | —— | —)— | —) | Regendanz and Reichenow (1932) |
| *Dermacentor pictus* Herm | Russia France | 3 | | X— | —) | | | | | Enigk (1944b) |
| *Dermacentor venustus* Banks = (*Dermacentor andersoni* Stiles) | France | 3 | | | X | - - | —— | —— | —) | Brumpt and Larrousse (1922) |
| *Haemaphysalis leachi* (Audouin) | South Africa | 3 | | | X— | - - | - - | —— | —) | Lounsbury (1901) |
| | | | | X— | —) | | | | | Brumpt (1938) |
| *Hyalomma marginatum* Koch | [illegible] | 3 | X | - - - | —— | —— | —— | —— | —) | Enigk (1944b) |
| *Rhipicephalus sanguineus* (Latreille) | India | 3 | | | X— | —— | —— | —)— | —) | Christophers (1907a, 1907b) |
| | | | X— | —) | | | | | | Shortt (1936) |
| | | | | X— | —) | | | | | |
| | Germany | | | | X— | —— | —)— | —)— | —) | Reichenow (1935) |
| | France | | | | X— | —— | —— | —— | —) | Brumpt (1919) |
| | South Africa | | | | X— | —— | —— | —— | —) | Neitz (1952) |
| | U.S.A. | | | | | | | | | Steinhaus (1947) |
| | Brazil | | | | | | | | | Regendanz and Muniz (1936) |

[illegible]

TABLE 10

THE BIOLOGICAL TRANSMISSION OF BABESIA GIBSONI BY MEANS OF TICKS

| Vectors | Country | No. of host | Larva | Nymph | Imago | Egg | Larva | Nymph | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Haemaphysalis bispinosa* Neum | India | 3 | | X→ | ) | | | | | Swaminath and Shortt (1937)<br>Shortt (1938) |
| | | | | | X→ | → | → ) | → ) | | |
| | | | | | X→ | → | → ) | | | |
| | | | X→ | ) | | | | | | |
| | | | | X→ | ) | | | | | |
| *Rhipicephalus sanguineus* (Latreille) | India | 3 | X→ | ) | | | | | | Sen (1933)<br>Datta (1940) |
| | | | | X→ | ) | | | | | |

(1929) was unable to transmit the disease with the common dog tick *Rh. sanguineus*. McNeil (1937) failed to transmit the infection with either larvae or nymphae derived from an adult female tick (*Haemaphysalis leachi*) that had engorged on a cat suffering from feline babesiosis. He, however, is of the opinion that the ensuing adults that had accidentally escaped from test tubes were responsible for transmitting the infection to his house cat.

### *Mechanical Transmission*

No conclusive evidence has been recorded in the literature where *Babesia* species have been transmitted mechanically by bloodsucking arthropods. Nevertheless this possibility must not be lost sight of, as it may follow if conditions are favorable.

### *Intra-Uterine Transmission*

A review on the intra-uterine transmission has been published by Enigk (1942). According to him, the factors directly responsible for this form of transmission have not yet been satisfactorily determined. One must assume that injuries to the placental blood vessels pave the way for piroplasms to migrate from the mother to the fetus. This form of transmission is of relatively rare occurrence, and hence it is of no significance for the general control of piroplasmosis. Nevertheless it should be taken into consideration in enzootic regions where it may be responsible for abortions. Research workers engaged in critical work on piroplasmosis in newborn animals should bear this phenomenon in mind.

The following intra-uterine transmissions have been recorded:

*B. bigemina* in calves has been recorded by Zolotareff (1936) and le Roux (1939).

*B. bovis* has been recorded in a fetus by Yakimoff (1936), and in calves by Waintraub (1938).

*B. ovis* has been established in two sheep fetuses by Donatien, Lestoquard and Kilcher-Maucourt (1934).

*B. caballi* has been diagnosed in a fetus by Donatien, Lestoquard and Bouguet (1935), and Martschenko (1936).

*B. equi* has been demonstrated in foals and fetuses by Carpano (1922), Donatien, Lestoquard and Sausseau (1924), Barnet (1931), Saint Martin (1933), Gauchot (1936), and Konstantinow (1936).

Mixed infection of *B. caballi* and *B. equi* has been seen in a fetus by Zolotareff (1936).

### *Artificial Transmission*

The various *Babesia* species can be readily transmitted to susceptible animals by the injection of blood and organ emulsions by the intravenous, subcutaneous, intraperitoneal, intramuscular, and other routes.

## Transmission of the Members of the Order Telcosporidea

### *Biological Transmission*

*Theileria* species of cattle:

*Theileria parva*. East Coast fever is one of the most important cattle dise-

in East Central and Southern Africa Its vectors are listed in TABLE 11 Seven *Rhipicephalus* species and three *Hyalomma* species have been proved experimentally to be transmitters *Rh appendiculatus* which is widely distributed in Africa is undoubtedly the chief vector Although the remaining *Rhipicephalus* species are less important they nevertheless play a significant role in maintaining the infection in nature

There is no evidence that *Hyalomma* species transmit the disease in nature The demonstration of Ray (1940 1941 1950) that *Hyalomma savignyi* can retain *Gonderia annulata* for five successive generations urges that investigations should be undertaken to determine whether or not any of the *Hyalomma* species can transmit *Th parva* hereditarily Should this prove to be the case an explanation may be found for the sudden sporadic outbreaks of East Coast fever three to five years after the last death from this disease in Southern Africa where systematic dipping and vigorous quarantine measures had been practised

Up-to-date stage to-stage transmission within the same generation only has been observed in the known vectors Another important feature of the arthropod transmission is the fact that infected nymphae lose their infection irrespective of whether they feed on a susceptible or unsusceptible animal

*Gonderia* species of cattle

*Gonderia annulata* The vectors of Mediterranean Coast fever (tropical theileriasis) are listed in TABLE 12 Six *Hyalomma* species are capable of transmitting the disease Stage to stage transmission within the same generation has been proved to occur in all of them Although Ray (1940 1941 1950) and Korienko and Shmuireva (1944) claim to have proved the hereditary transmission of *G annulata* in *H savignyi* and Korienko and Shmuireva (1944) in *H Turkmeniense* (= *H excavatum* Delpy (1949) states that there is no evidence of this form of transmission of this parasite by the mother to its progeny It is of interest to note that *G annulata* cannot be transmitted by *Rhipicephalus* species

*Gonderia mutans* The ticks responsible for the transmission of Tzaneen disease (*G mutans* infection benign gonderiosis) are listed in TABLE 13 In the case of the two *Rhipicephalus* species stage to-stage transmission within the same generation occurs Reichenow (1935) states that Miessner obtained *B annulatus* ticks from the United States of America and succeeded in transmitting *G mutans* with their progeny in Germany The recent demonstration of *G mutans* in Europe (England) suggests that Miessner s observation must be confirmed before it can be accepted without reservation that *B annulatus* is a vector of this protozoon

*Gonderia laurencei* It has recently been established that the vector of *G laurencei* which is responsible for Corridor disease is *Rh appendiculatus* (TABLE 14) Larvae and nymphae that fed on a buffalo calf harboring the infection transmitted the protozoon in the ensuing stages

*Gonderia* species of sheep and goats

*Gonderia ovis* It will be seen from TABLE 15 that *G ovis* can be transmitted by two *Rhipicephalus* species The infection is acquired by the larvae and

TABLE 11

THE BIOLOGICAL TRANSMISSION OF THEILERIA PARVA BY MEANS OF TICKS

| Vector | Country | No. of host | Larva | Nymph | Imago | Egg | Larva | Nymph | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Rhipicephalus appendiculatus* Neum.<br>Syn. (*Rhipicephalus nitens* Neum.) | Central East and Southern Africa | 3 | X—— | ——)<br>X—— | ——) | | | | | Lounsbury (1903b)<br>Theiler (1905a, b, c)<br>Montgomery (1913)<br>Fotheringham and Lewis (1937) |
| *Rhipicephalus ayrei* Lewis = ?(*Rhipicephalus compositus* Neum.) | East and Central Africa | 3 | X—— | ——)<br>X—— | ——) | | | | | Wilson (1953) |
| *Rhipicephalus capensis* Koch | Southern Africa | 3 | X—— | ——)<br>X—— | ——) | | | | | Lounsbury (1906) |
| *Rhipicephalus evertsi* Neum. | Central East and Southern Africa | 2 | X—— | ——X—— | ——) | | | | | Lounsbury (1906)<br>Theiler (1906c)<br>Fotheringham and Lewis (1937) |
| *Rhipicephalus jeanelli* Neum. = (*Rhipicephalus kochi* Dönitz) | East and Central Africa | 3 | X—— | ——) | | | | | | Wilson (1953) |
| *Rhipicephalus neavei* Warburton = (*Rhipicephalus tricuspis punctatus* Warburton = *Rhipicephalus pravus* Dönitz) | East Africa | 3 | X—— | ——)<br>X—— | ——) | | | | | Lewis, Piercy and Wiley (1946) |
| *Rhipicephalus simus* Koch | South and East Africa | 3 | X—— | ——)<br>X—— | ——) | | | | | Theiler (1906a, b, c)<br>Lounsbury (1906)<br>Fotheringham and Lewis (1937)<br>Neitz and Jansen (1950) |
| *Hyalomma anatolicum* Koch = (*Hyalomma excavatum* Koch according to Delpy and Feldman in Muhsam 1954) | East Africa (Laboratory observations) | 3 | X—— | ——)<br>X—— | ——) | | | | | Lewis and Fotheringham (1941) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Hyalomma dromedarii* Koch | East Africa (laboratory observations) | 3 | X— | —) X— | —) | | | | | Lewis and Fotheringham (1941) |
| *Hyalomma impressum* near *planum* Lewis = (? *Hyalomma transiens* Schulze = *Hyalomma truncatum* Koch) | East Africa (laboratory observations) | 2 or 3 | X— | —) X— | —) | | | | | Fotheringham and Lewis (1937) |

TABLE 12
THE BIOLOGICAL TRANSMISSION OF GONDERIA ANNULATA BY MEANS OF TICKS

| Vectors | Country | No. of hosts | L | Nympha | Imago | Egg | La | Nymph | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Hyalomma detritum* Schulze Syn. (*Hyalomma mauritanicum* Senevet) | North Africa | 2 | ×— | —×— | —) | | | | | Sergent, Donatien, Parrot and Lestoquard (1928) |
| | Russia | | ×— | —×— | —) | | | | | Galuzo (1935) Galuzo and Bespalov (1935) |
| *Hyalomma dromedarii* Koch Syn. (*Hyalomma dromedarii asiaticum* Schulze and Schlottke according to Delpy 1949b = *Hyalomma dromedarii* Koch according to Feldman-Muhsam 1954) | Central Asia | 2 | ×— | —×— | —) | | | | | Galuzo (1934) |
| *Hyalomma excavatum* Koch Syn. (*Hyalomma anatolicum* Koch according to Delpy 1949b and Feldman-Muhsam 1954) | Asia Minor | 3 | ×— | —) ×— | —) | | | | | Delpy (1949a) |
| *Hyalomma impressum near planum* Lewis (= ? *Hyalomma transiens* Schulze according to G. Theiler = *Hyalomma truncatum* Koch according to Feldman-Muhsam 1954) | East Africa (Laboratory observations) | 2 or 3 | | ×— | —) | | | | | Fotheringham and Lewis (1937a) |
| *Hyalomma rufipes glabrum* Delpy (= *Hyalomma turanicum* Pomerantzev) | Asia Minor | 2 | ×— | —×— | —) | | | | | Delpy (1949a) |
| *Hyalomma marginatum* (Gervais) (= *Hyalomma marginatum* Koch according to Feldman-Muhsam 1954) | Asia Minor | 2 or 3 | | ×— | —) | | | | | Delpy (1949a) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Hyalomma savignyi* (Gervais) (Referred to originally as *Hyalomma aegyptium* Neum but according to Ray (1950) identified by Adler and Feldman-Muhsam as *Hyalomma savignyi* (Gervais)) | India<br><br>(Hereditary transmission through four generations) | 2 or 3 | <br><br>—<br>— | <br><br>—<br>— | X—<br>X—<br>—)—<br>—) | —<br>—<br>— | —<br>—<br>— | —<br>—<br>— | —)<br>—)<br>—) | Ray (1940-41)<br>Raghavachari Shah and Ray (1944)<br>Kornenko and Shmureva (1944)<br>Ray (1950) |

TABLE 13

THE BIOLOGICAL TRANSMISSION OF GONDERIA MUTANS BY MEANS OF TICKS

| Vectors | Country | No. of host | Larva | Nymph | Imago | Egg | Larva | Nymph | Imago | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Boophilus annulatus* (Say) | Germany with ticks from United States of America | 1 | ?<br>X— | ?<br>—X— | ?<br>—X— | — | ?<br>—)— | ?<br>—)— | ?<br>—)— | Miessner according to Reichenow (1935) |
| *Rhipicephalus appendiculatus* Neum | South Africa | 3 | <br>X— | X—<br>—) | —) | | | | | Theiler (1909a)<br>Neitz and Jansen (19-0)<br>Neitz (1938) |
| *Rhipicephalus evertsi* Neum | South Africa | 2 | X— | —X— | —) | | | | | Theiler (1909a) |

TABLE 14

THE BIOLOGICAL TRANSMISSION OF GONDERIA LAWRENCEI BY MEANS OF TICKS

| V[ector] | C[o]u[n]try | N[o. o]f [s]h[if]ts | L[arva] | Nympha | Imag[o] | Egg | La[rv]a | Nym[p]h[a] | Im[a]g[o] | Ref[erence]s |
|---|---|---|---|---|---|---|---|---|---|---|
| *Rhipicephalus appendiculatus* Neum | South Africa | 3 | X— | —)<br>X— | <br>—) | | | | | Neitz (1955a)<br>Neitz Canham & Kluge 1955) |

TABLE 15

THE BIOLOGICAL TRANSMISSION OF GONDERIA OVIS BY MEANS OF TICKS

| Vect[or] | C[oun]t[r]y | N[o. o]f [s]h[if]t[s] | La[rva] | Nym[p]h[a] | Im[a]g[o] | Egg | La[rva] | Nym[p]h[a] | Im[a]go | R[e]f[eren]c[e] |
|---|---|---|---|---|---|---|---|---|---|---|
| *[illegible]ipicephalus bur[sa]* Canestrini and Fanzago | [R]u[s]sia | 2 | X— | —X— | —) | | | | | Pa[illegible] tegaieff (1933 1934) |
| *Rhipicephalu[s] [illegible]e[illegible]e tsi* Neum | South Africa | 2 | X— | —X— | —) | | | | | Jansen and Neitz (19[illegible]5) |
| *Ornit[ho]d[o]rus lah[o]ren[si]s* Neum | Ru[ss]ia | Many | | | —) | | | | | [illegible]astigaieff (1935) |

nymphae and is given off by the imago Rastegaieff (1933) claims to have transmitted *G ovis* with *Ornithodorus lahorensis* If this observation is correct then this is the first record that a piroplasm is transmitted by a member of the family Argasidae

*Gonderia hirci* The vector of *G hirci* has not yet been established It is possible that *Rhipicephalus bursa* is a vector in the enzootic areas

### *Mechanical Transmission*

No conclusive evidence has been recorded in the literature where *Theileria* species and *Gonderia* species have been transmitted mechanically by blood sucking arthropods

### *Intra Uterine Transmission*

Information on the intra uterine transmission in cattle and sheep is limited Sergent Donatien and Parrot (1924) record the presence of *G annulata* in a fetus Sprinzholz Schmidt (1937b) demonstrated a *Gonderia* species (*Theileria sergenti*) in a fetus Magneville (1925) observed a *Gonderia* species in a seven day-old calf Lestoquard (1926a) found *Theileria ovis* (= *G hirci*) in two lambs

### *Artificial Transmission*

*Theileria parva* of cattle The artificial transmission of *Th parva* by the injection of blood can be achieved in relatively few cases K F Meyer (1909) however showed that the intravenous injection of an emulsion prepared from the spleen and glands produced symptoms of this disease in a relatively large number of animals With this method of infection it was shown that there is a striking difference between the naturally and artificially produced diseases In the former the course of the disease is fatal in 95 per cent of cases whereas in the latter more than 50 per cent of the animals that react recover The observations of K F Meyer (1909) were confirmed on a very large number of cattle by Theiler (1912) The results showed that the course and nature of the reactions following an artificial infection with spleen and gland emulsions was markedly different from that observed in cattle that acquired the disease naturally The different types of reaction in the artificially infected cattle may be briefly summarized as follows

(1) Typical reactions with a fatal termination were observed in 24 3 per cent of cattle (*Th parva* in the erythrocytes and Koch bodies could be demonstrated)

(2) Typical reactions followed by recovery were noticed in 27 3 per cent of the animals (*Th parva* in the erythrocytes and Koch bodies could be demonstrated)

(3) Mild reactions developed in 13 per cent of the cattle which recovered (*Th parva* in the erythrocytes and Koch bodies could not be demonstrated)

(4) Irregular reactions from which the animals recovered occurred in 19 2 per cent of cattle (*Th parva* in the erythrocyte and Koch bodies could not be demonstrated)

(5) In 21 per cent of cattle no reactions and no parasites were observed at all The animals that survived the artificial infection were all subjected to an immunity test by infesting them with known infected ticks The results of the e tests were surprising Generally speaking it can be stated that the pattern of the different types of reactions in each group following the immunity tests had some resemblance to that exhibited originally by the susceptible cattle after the artificial infection Deaths occurred in all groups irrespective of whether typical atypical or no reactions had been noticed previously What is even more striking is that as many as 46 7 per cent of the cattle that had not reacted at all to the artificial infection were found to be either partially or even solidly immune when challenged

In a second series of experiments Theiler and du Toit (1928 1929) attempted to transmit East Coast fever with infective blood or emulsions prepared from partially engorged infected *Rh appendiculatus* ticks The results of these experiments were similar to those observed previously Typical atypical and no reactions were seen Immunity tests applied on several of the animals revealed the presence of a partial or a complete immunity even in such cases that had not reacted to the artificial infection Consideration of these results show that the nature of the immunity in East Coast fever is not in accordance with our present accepted views on protozoal immunity in general The suggestion was advanced by Neitz (1943) that East Coast fever is a combined infection of at least two factors namely a protozoal parasite *Th par a* and probably a virus

*Gonderia* species of cattle

*Gonderia annulata* *G annulata* can be maintained by serial passage in cattle provided subinoculations of blood and organ emulsions are carried out during the course of the febrile reaction According to Cordier Menager and Delorme (1936) passage of the Kouba strain produced a remarkable change in the developmental cycle of *G annulata* After several generations only Koch bodies appeared but no parasites in the erythrocytes Sergent Donatien Parrot and Lestoquard (1932) state that all attempts to infect the vector with the vaccine strain of *G annulata* have failed The inference is drawn that this strain can be safely used for the immunization of cattle without the danger of creating reservoirs for the infection of vectors

*Gonderia mutans* The artificial infection of cattle with infective blood (Theiler and Graf 1928) and with infective organ emulsions and blood (de Kock van Heerden du Toit and Neitz 1937) resulted in the appearance of only erythrocytic parasites of *G mutans* All attempts to demonstrate Koch bodies failed The inability to demonstrate schizonts does not necessarily mean that this stage of the parasite had not developed in the host Nevertheless Neitz (1952) demonstrated that the erythrocytic stages can multiply and maintain themselves for months in the complete absence of schizonts Blood from a calf harboring a pure infection of *G mutans* was injected into a fully susceptible splenectomized calf Four weeks later parasites appeared in the peripheral circulation Clean *Rh appendiculatus* nymphae were allowed to feed on this animal Three months after moulting the ears of this calf were

reinfested with the ensuing adults Twelve days later this animal developed a thermal reaction and Koch bodies could be demonstrated in the markedly swollen parotid gland and in the moderately swollen prescapular and precrural lymphatic glands This experiment was repeated with the same results in two more splenectomized calves The inference is drawn that the erythrocytic stages of *G mutans* can maintain themselves for long periods in the complete absence of schizonts and that the latter stage of the protozoon confers the immunity

*Gonderia lawrencei* The attempts at transmitting *C lawrencei* with blood and organ emulsions collected from an ox suffering from Corridor disease to two susceptible calves failed (Neitz Canham and Kluge 1955) This work must be repeated before final conclusions can be made

*Gonderia* species of sheep and goats

*Gonderia hirci* Du Toit (1930) states that *G hirci* can be easily transmitted with infective blood The disease is transmissible from sheep to goats and *vice versa*

*Gonderia ovis* Lestoquard (1929) infected a young goat with sheep s blood containing *G ovis* and then splenectomized the animal It was thereupon infected with cultures of paratyphoid B Koch bodies appeared in its glands and the erythrocytic parasites were demonstrable in the peripheral circulation

## The Biological Properties of the Piroplasms

It is evident from the above discussion that apart from the morphology certain biological properties namely the cyclical transmission by ticks the pathogenicity and the immunological properties of the piroplasms have been of extremely great assistance to the protozoologist in differentiating between the numerous species The knowledge of these characteristics is of equally great importance to the veterinarian who is entrusted with the control of the diseases and to the chemotherapeutist engaged in the synthesis of specific drugs It is essential that these investigators know how these protozoa maintain themselves in nature and whether chemotherapy for the treatment of naturally infected animals and for the control of reactions during the process of immunization or alternatively whether the destruction of infected vectors will be the correct approach for the control of tick borne diseases The latter procedure has almost completely eradicated babesiosis in the United States of America and East Coast fever in Southern Africa These results are both achievements of significant importance

### *Pathogenicity*

The domestic and wild animals susceptible to certain piroplasms are listed in the appended TABLES 16 to 24 The writer wishes to thank R Bigalke Director of the National Zoological Gardens Pretoria Union of South Africa for his kind assistance in determining the correct zoological names of the susceptible wild animals

From the available information one is justified in concluding that the piroplasms are stenoxenous parasites *B caballi* not included in the appended

TABLE 16

DOMESTIC AND WILD MEMBERS OF THE FAMILY BOVIDAE SUSCEPTIBLE TO BABESIA BIGEMINA

| Host | | Country | Ol t | Reference |
|---|---|---|---|---|
| Vernacular Name | Zoological Name | | | |
| Cattle, European, Asiatic and African breeds, including the Zebu cattle | *Bos* spp. | Africa, America, Asia, Australia and Southern Europe | Naturally and artifically infected | Knuth and du Toit (1921)<br>Reichenow (1953) |
| Water buffalo | *Bubalus bubalis* (Linn.) | Egypt | Naturally infected | Knuth and du Toit (1921)<br>Littlewood (1922)<br>Lalaghatti (1926) |
| Deer | *Mazama americana reperticia* Goldman (= *Mazama sartorii reperticia* J. A. Allen) | Panama | Naturally infected | Clark and Zetek (1925) |
| White-tailed deer | *Odocoileus virginianus chiriquensis* J. A. Allen (= *Odocoileus chiriquensis* J. A. Allen) | Panama | Naturally and artificially infected | Clark (1918)<br>Clark and Zetek (1925) |

TABLE 17

DOMESTIC AND WILD MEMBERS OF THE FAMILY EQUIDAE SUSCEPTIBLE TO BABESIA EQUI

| Host | | Country | [illegible] | References |
|---|---|---|---|---|
| Vernacular Name | Zoological Name | | | |
| Horse | *Equus caballus* Linn. | Africa, Asia, Europe | Naturally and artificially infected | Laveran (1901)<br>Knuth and du Toit (1921) |
| Mule | *Equus asinus* × *Equus caballus* | South Africa<br>European Russia | Naturally infected | Theiler (1905c, 1906c)<br>Dschunkowsky and Luhs (1909) |
| Donkey | *Equus asinus* Linn. | South Africa | Naturally and artificially infected | Dale (1903)<br>Theiler (1905c, 1906c)<br>Jansen (1951) |
| Zulu Burchell's zebra | *Equus burchelli wahlbergi* (Pocock) (= *Hippotigris quagga* Pocock) | South Africa<br>Zululand | Naturally infected | Theiler (1909b)<br>Neitz (1931, 1933) |
| Burchell's zebra | *Equus burchelli granti* de Winton | Uganda<br>East Africa | Naturally infected | Ross (1907, 1911)<br>Kuhlicke (1912) |

TABLE 18
DOMESTIC AND WILD MEMBERS OF THE FAMILY CANIDAE SUSCEPTIBLE TO BABESIA CANIS

| Host | | Country | Observations | References |
|---|---|---|---|---|
| Vernacular Name | Zoological Name | | | |
| Dog | *Canis familiaris* Linn | Africa America Asia Europe | Naturally and artificially infected | Piana and Galli Valerio (1895)<br>Knuth and du Toit (1921)<br>Wenyon (1926)<br>Laugh (1944b)<br>Reichenow (1953) |
| Jackal | *Canis lupaster algirensis* Wagner | Algeria | Artificially infected | Gayot (1946) |
| Wolf | *Canis lupus* Linn | Turkestan | Naturally infected | Yakimoff and Schokhor (1917) |
| Red fox<br>Silver fox | *Canis vulpes* Linn (*Vulpes vulgaris* Oken) | Germany | Artificially infected | Schoop and Dedié (1938) |
| Side-striped jackal | *Thos adustus* (Sundevall) (*Canis adustus* Sundevall) | East Africa | Naturally infected | Nuttall (1910) |
| Black-backed jackal | *Thos mesomelas mesomelas* (Schreber) | South Africa | Naturally and artificially infected | Neitz and Steyn (1947) |

TABLE 19

DOMESTIC AND WILD MEMBERS OF THE FAMILY CANIDAE SUSCEPTIBLE TO BABESIA GIBSONI

| Host | | Country | Observation | Reference |
|---|---|---|---|---|
| Vernacular Name | Zoological Name | | | |
| Dog | *Canis familiaris* Linn | India | Naturally and artificially infected | Patton (1910) |
| Jackal | *Canis aureus* Linn | India | Naturally and artificially infected | Patton (1910) |
| Wolf | *Canis lupus* Linn | Turkestan | Naturally infected | Yakimoff and Schokhor (1917) |
| Indian wild dog | *Cuon dukhunensis* (Sykes) = (*Cyon dukhunensis* (Sykes)) | London Zoological Gardens | Naturally infected | Plimmer (1915) |
| Fox | *Vulpes vulpes dorsalis* (Gray) = (*Canis vulpes dorsalis* Gray, *Fennecus dorsalis*) | French Sudan | Naturally infected (*Nuttallia bauryi* possibly *B. gibsoni*) | Léger and Bédier (1922) |
| Fox | Zoological name not mentioned | Russia | Naturally infected (*Theileria* species possibly *B. gibsoni*) | Yakimoff and Schokhor (191[illegible]) |

TABLE 20
DOMESTIC AND WILD MEMBERS OF THE FAMILY FELIDAE SUSCEPTIBLE TO BABESIA FELIS

| Host | | Country | Observations | References |
|---|---|---|---|---|
| Vernacular Name | Zoological Name | | | |
| Domestic cat | *Felis catus* Linn | Egyptian Sudan | Artificially infected<br>Mild reactions | Davis (1929) |
| | | Union of South Africa | Naturally infected<br>Relatively mild and fatal reactions | Parkin (1927)<br>Jackson and Dunning (1937)<br>McNeil (1937)<br>Brownlie (1954) |
| American puma Cougar mountain lion | *Felis concolor* Linn (= *Felis concolor*) | Zoological Gardens Cairo Egypt | Naturally infected | Carpano (1934) |
| Sudanese lion | *Felis leo* Linn | French Sudan | Naturally infected | Léger and Bédier (1922) |
| Sudanese wild cat Lybian cat Fettered cat | *Felis lybica ocreata* J F Gmelin (= *Felis ocreata* Gmelin) | Egyptian Sudan | Naturally infected | Davis (1929) |
| American lynx Bobcat | *Lynx rufus* (Schreber)(= *Felis rufa*) | Zoological Gardens London | Naturally infected | Wenyon and Hamerton (1930) |
| Indian leopard | *Panthera pardus fusca* (Meyer) | Naturally infected *Babesia* species possibly *B felis* | | Shortt (1940) |

TABLE 21

DOMESTIC AND WILD MEMBERS OF THE FAMILY BOVIDAE SUSCEPTIBLE TO THEILERIA PARVA

| Host: Vernacular Name | Host: Zoological Name | Country | Observation | Reference |
|---|---|---|---|---|
| Cattle | *Bos taurus* Linn. | East, Central and Southern Africa | Naturally and artificially infected | Koch (1898)<br>Theiler (1904) |
| African buffalo | *Syncerus caffer* Sparrman | East Africa | Naturally infected | Lewis (1913) |
| Indian water buffalo | *Bubalus bubalis* Linn. | East Africa<br>South Africa | Naturally infected<br>Naturally infected | Bradshaw (1924)<br>Neitz (1940) |

TABLE 22

DOMESTIC AND WILD MEMBERS OF THE FAMILY BOVIDAE SUSCEPTIBLE TO GONDERIA ANNULATA

| Host: Vernacular Name | Host: Zoological Name | Country | Observation | Reference |
|---|---|---|---|---|
| Cattle: European, Asiatic and African breeds including the Zebu cattle | *Bos spp.* | North Africa, Asia, South Eastern Europe | Naturally and artificially infected | Dschunkowsky and Luhs (1904)<br>Knuth and du Toit (1921)<br>Sergent *et al.* (1924a, b) |

TABLE 20

DOMESTIC AND WILD MEMBERS OF THE FAMILY FELIDAE SUSCEPTIBLE TO *BABESIA FELIS*

| Host | | Country | Observations | References |
|---|---|---|---|---|
| Vernacular Name | Zoological Name | | | |
| Domestic cat | *Felis catus* Linn | Egyptian Sudan | Artificially infected<br>Mild reactions | Davis (1929) |
| | | Union of South Africa | Naturally infected<br>Relatively mild and fatal reactions | Larkin (1927)<br>Jackson and Dunning (1937)<br>McNeil (1937)<br>Brownlie (1954) |
| American puma (Cougar, mountain lion) | *Felis concolor* Linn (= *Felis concolor*) | Zoological Garden, Cairo, Egypt | Naturally infected | Carpano (1934) |
| Sudanese lion | *Felis leo* Linn | French Sudan | Naturally infected | Léger and Bédier (1922) |
| Sudanese wild cat (Egyptian cat, Kaffir cat) | *Felis lybica ocreata* J. F. Gmelin (= *Felis ocreata* Gmelin) | Egyptian Sudan | Naturally infected | Davis (1929) |
| American lynx (bobcat) | *Lynx rufus* (Schreber) (= *Felis rufa*) | Zoological Gardens, London | Naturally infected | Wenyon and Hamerton (1930) |
| Indian leopard | *Panthera pardus fusca* (Meyer) | Naturally infected *Babesia* species possibly *B. felis* | | Shortt (1940) |

tables is known to be transmissible from horses to mules and donkeys The host range of *B bovis B argentina B berbera B major* of cattle and that of *B trautmanni* and *B perroncitoi* of swine needs to be determined The sporadic appearance of *B trautmanni* in Africa in pigs introduced from nonenzootic regions suggests that the wart hog (*Phacochoerus* sp) and bush pig (*Potamochoerus* sp) act as carriers of this protozoon

### *Immunity*

Studies on diseases caused by piroplasms have shown that with the exception of East Coast fever a complete or absolute immunity does not occur in any of them

*Immunity produced by members of the suborder Piroplasmidea* Recovery from babesiosis confers on animals a fairly strong resistance or tolerance to a subsequent infection with the same immunological strain The existence of immunologically different strains has been determined under laboratory conditions and in nature The transference of animals from one enzootic area to another may be followed by a second attack of babesiosis The primary attack confers a variable degree of protection against a subsequent reinfection and depending upon the immunogenic properties and virulence of the superimposed *Babesia* species mild or alternatively severe reactions that may terminate fatally can be expected This type of reaction must be differentiated from relapses following infections with other diseases caused by bacteria rickettsiae protozoa viruses and helminths

The immunity in babesiosis is referred to as a premunition labile infection latent infection or *immunitas non sterilisans* This persists as long as the animal remains a carrier of the infection A latent infection may be harbored for periods of up to two years as shown in cattle sheep swine and dogs or alternatively for longer periods of up to 12 years as demonstrated in cattle after recovery from a *B bigemina* infection Parasites may disappear completely as a result of autosterilization or after repeated treatment with specific drugs Disappearance of the infectious agent renders animals fully susceptible

It has been the experience of numerous investigators that with the exception of puppies calves and foals under the age of a year and of lambs kids and piglets up to the age of three months are highly resistant against *Babesia* infections (no information is available about the resistance of kittens) A satisfactory explanation has not yet been offered for this phenomenon The only anatomical and physiological difference between these animals is that in puppies involution of the thymus commences within a few days after birth while in the other species this organ persists for several months before degeneration sets in It needs to be determined whether or not the thymus is responsible for this resistance

The immunity in babesiosis may be interrupted when animals suffer from an intercurrent infection or after splenectomy It is generally accepted that the reticuloendothelial system participates in the battle of protection Phagocytosis by monocytes prevents an unlimited increase in the parasites and maintains the labile balance

TABLE 23

DOMESTIC AND WILD MEMBERS OF THE FAMILY BOVIDAE SUSCEPTIBLE TO GONDERIA MUTANS

| Host | | Country | Observations | References |
|---|---|---|---|---|
| Vernacular Name | Zoological Name | | | |
| Cattle | *Bos taurus* Linn | Africa<br><br>North America<br>Asia<br>Australia<br>Europe<br>(England) | Naturally and artificially infected | Theiler (1906b)<br>Knuth and du Toit (1921)<br>Spliter (1950)<br>Knuth and du Toit (1921)<br>Legg (1935)<br>Knuth and du Toit (1921)<br>Hignett (1953) |
| African buffalo | *Syncerus caffer* Sparrman | East Africa<br>South Africa | Artificially infected | Walker (1932)<br>Neitz (1940) |

TABLE 24

DOMESTIC AND WILD MEMBERS OF THE FAMILY BOVIDAE SUSCEPTIBLE TO GONDERIA LAWRENCEI

| Host | | Country | Observations | References |
|---|---|---|---|---|
| Vernacular Name | Zoological Name | | | |
| Cattle | *Bos taurus* Linn | Southern Rhodesia<br>South Africa | Naturally infected | Lawrence (1934)<br>Neitz (1955a)<br>Neitz Canham & Kluge (1955) |
| African buffalo | *Syncerus caffer* Sparrman | Zululand<br>Eastern Transvaal | Naturally infected | Neitz (1955a) |

1937) From this observation it is deduced that the demonstration of erythrocytic parasites in a naturally infected animal does not necessarily mean that it is still harboring schizonts It merely signifies that it has been exposed to *G mutans* infected ticks at some stage of its life The importance of conducting similar experiments on *G annulata G hirci* and *G ovis* is self evident

Immunization against Mediterranean Coast fever is practised in North Africa and in Israel For this purpose a mild strain of the local organism is employed and it is emphasised that it should be done in calfhood (Richardson 1948)

The resistance of young animals to *Theileria* and *Gonderia* infections appears to be less pronounced than in the case of babesiosis The mortality from amakebe presumably *Th parva* infection in Tanganyika varies from 10 to 30 per cent The writer found in South Africa that calves up to the age of three weeks were as susceptible to East Coast fever as adult stock Machattie (1935) recorded a mortality rate of 50 per cent due to *G annulata* in Bagdad while in other enzootic areas calves appear to be highly resistant

## Summary and Conclusion

(1) A historical review of the classification of the piroplasms including the classificatory lists compiled by earlier workers are given

(2) Reasons are offered for the revision of the previous classifications

(3) Consideration of the striking differences between the life cycles of the Babesidae and those of the Theileridae and Gonderidae suggests that the former family should be retained in the suborder Piroplasmidea Wenyon 1926 and that the latter two families be included in the suborder Leucosporidea Neitz and Jansen 1956

(4) Both suborders are retained in the order Coccidia of the class Sporozoa

(5) The revision of the classification is based on the fact that members of the Piroplasmidea multiply by binary fission within the erythrocytes while members of the Leucosporidea reproduce by schizogony in the leukocytes

(6) The suborder Piroplasmidea is represented by a single family Babesidae a single genus *Babesia* and a large number of species

(7) The suborder Leucosporidea is represented by two families Theileridae and Gonderidae A single genus and species *Theileria parva* is retained in the Theileridae The family Gonderidae is represented by two genera *Gonderia* and *Cytauxzoon* comprising many species

(8) The member of the family Theileridae multiplies only within the lymphocytes by schizogony while members of the family Gonderidae reproduce by schizogony within the lymphocytes (*Gonderia* spp) or within the histiocytes (*Cytauxzoon* sp) and by binary fission in the erythrocytes

(9) Two classificatory lists one of the Piroplasmidea and another one of the Leucosporidea are given

(10) The known arthropod vectors of the piroplasms are detailed in a series of tables

(11) Mention is made of the occurrence of the intra uterine transmission of the piroplasms

Numerous experiments have shown that there is no cross immunity between the different *Babesia* species. Relapses may develop in animals immune to one species while reacting to another.

Animals can be rendered immune to babesiosis by infecting them artificially with the infectious agent and treating the ensuing reaction with specific drugs. This method of immunization is being practised in cattle in several countries with satisfactory results. In enzootic areas young animals acquire an immunity that renders them resistant to subsequent infections. The state of premunition is maintained by repeated reinfestation by infected ticks.

*Immunity produced by members of the suborder Leucosporidea.* Certain aspects of the immunity exhibited by the *Theileria* species and *Gonderia* species have already been referred to in the previous two subheadings and should be read in conjunction with this discussion.

Cattle that recover from a naturally acquired East Coast fever reaction develop a solid immunity that persists for a variable length of time. This immunity may commence to wane after a year and may disappear completely after a few years. The writer has observed that in some cases the immunity was still solid after five years. When testing the immunity of recovered animals, various results can be expected. Sometimes no reactions are observed at all. Then again either very mild reactions develop during which only Koch bodies appear in the superficial lymphatic glands, or moderate reactions are seen during which Koch bodies and erythrocytic parasites appear. Ticks are capable of infecting themselves while feeding on animals showing the latter type of reaction, and of transmitting the virulent form of East Coast fever. Such moderately affected animals escape detection and are liable to complicate the East Coast fever eradication scheme as practiced in South Africa.

The immunization of cattle by means of the intravenous administration of spleen and gland suspensions has been practised for only a short period in South Africa. It proved unsatisfactory, since as many as 25 per cent of animals died during the course of immunization, and of the survivors not more than 60 to 70 per cent proved to be immune on being exposed to tick infestation. The immunization as a control measure was superseded by systematic dipping in arsenical solutions.

Recently Neitz (1953) has shown that cattle can be immunized successfully against East Coast fever. The infection following the infestation with infected ticks is suppressed by repeated intravenous administration of Aureomycin (10 mg./kg.) on alternate days during the incubation period and during the first few days of the reaction. Although this immunization process cannot be applied in practice, it is nevertheless believed that it will be of assistance in immunological studies at laboratories.

Animals that have recovered from infections due to the different *Gonderia* species develop an immunity. The erythrocytic parasites persist throughout life. It has not yet been determined whether or not the schizonts are retained for the same period. In the case of *G. mutans* it has been determined that no immunogenic relationship exists between the schizonts and the erythrocytic stage of the parasite (Neitz, 1952). Moreover, the erythrocytic parasites can maintain themselves in cattle in the complete absence of schizonts (Neitz

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(12) The available information on the pathogenicity of the piroplasms is detailed in a series of tables

(13) The data show that the piroplasms are stenoxenous parasites

(14) The nature of the immunity exhibited by the piroplasms is briefly discussed

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fission or by budding with never more than four merozoites being produced The latter invariably become arranged in a rosette or in a crosslike formation

On the basis of general nuclear and cytoplasmic morphology staining reaction constancy in the number and configuration of the merozoites it is now proposed to include *D jahni* and *D mariae* in a new genus to which the name *Babesiosoma* gen nov is given The name *Babesiosoma* is selected because of the striking similarity to *Babesia quadrigemina*

*Morphology and Life History of* Dactylosoma

According to Noller (1913) the asexual stage of *Dactylosoma ranarum* is an elongated or rounded form which may be seen within the living erythrocyte as a hyaline mass of protoplasm containing refringent globules Giemsa stained preparations show trophozoites with 4 to 16 distinct nuclei the latter usually arranged at the periphery Schizogony gives rise to 4 to 16 merozoites with spherical nuclei arranged in a fanlike pattern A second type of schizogony gives rise to merozoites with slightly elongated or dumbbell shaped nuclei These become gametocytes with spherical nuclei the male gametocytes possess small karyosomes and the female gametocytes have large karyosomes Noller was unable to obtain transmission with the leech *Hemiclepsis marginata* which is a known vector for trypanosomes and other blood parasites of European frogs and fish In view of these negative results Noller suggested that the fish louse *Argulus foliaceus* is the probable vector We agree with Wenyon (1926) that the reason for this suggestion is not clear especially since it is known that these copepods are not bloodsucking organisms

Similar developmental stages have been described for *Dactylosoma salvelini* from the brook trout *Salvelinus fontinalis* and for *D sylvatica* from the wood frog *Rana sylvatica* by Fantham Porter and Richardson (1942) In *D salvelini* the youngest stage observed was a small oval or round body with a round nucleus and a distinct karyosome Binucleate tetranucleate and schizonts with 6 7 and 8 nuclei were found Most of the mature schizonts were fan to wedge shaped with nuclei arranged on two sides or peripherally In some cytoplasmic segmentation to form merozoites was indicated in others merozoites were formed and in a few cases completely separated merozoites were seen scattered in the cytoplasm of the erythrocytes Two types of gametocytes were also observed One was oval or reniform with granular cytoplasm and a rounded nucleus with rather dense chromatin The second type was also reniform It had a much paler staining cytoplasm and a nucleus of scattered chromatin granules By comparison with other Haemosporidia and their staining reaction Fantham Porter and Richardson were inclined to consider forms with granular cytoplasm and denser chromatin as macrogametocytes and those with paler staining cytoplasm and granular nuclei as microgametocytes Further stages in development were not found

The schizont schizogony and number of merozoites produced in *D sylvatica* were similar to those seen in *D salvelini* differing mainly in dimensions and host specificity Forms interpreted as male and female gametocytes were also found distinguished on the same basis as the gametocytes of *D salvelini* But

# *BABESIOSOMA* GEN NOV AND OTHER BABESIOIDS IN ERYTHROCYTES OF COLD BLOODED VERTEBRATES

*By* Sophie Jakowska and Ross F Nigrelli
*College of Mount St Vincent and New York Zoological Society New York N Y*

At present it is amongst the Haemo poridia of cold blooded Vertebrata that researches are most needed —F A Minchin (1903)

## *Introduction*

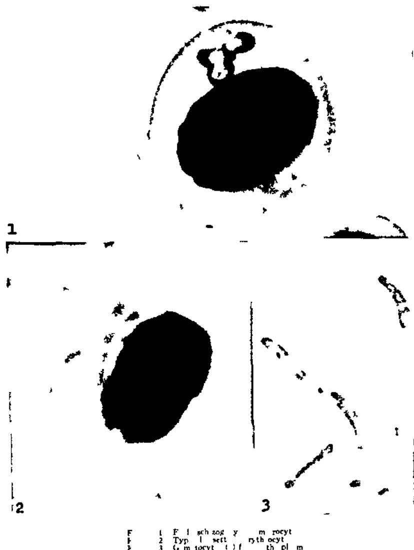
The history of our knowledge of blood inhabiting protozoans is intimately connected with their discovery in cold blooded vertebrates Numerous important firsts ' have originated from the interest of the early investigators in parasites of fish amphibians and aquatic reptiles Thus the first observation of a blood parasite was that of Valentin (1841) who saw trypanosomes in trout Mammalian trypanosomes were not discovered until 25 to 30 years later Also the first blood inhabiting sporozoan in a poikilothermic vertebrate was reported in 1871 by Lankester This parasite was later designated *Dactylosoma ranarum* now recognized as a nonpigmented haemosporidian This finding together with the discovery of the malarial parasite by Laveran (1880) is credited with laying the foundation to our knowledge of the Haemosporidia It apparently excited such interest in the early 20th century that in the words of Minchin (1903) the study of Sporozoa was to assume in the near future a position of importance scarcely secondary to that held by the science of bacteriology

## *Babesioids in Cold Blooded Vertebrates*

Wenyon (1926) suggested that certain unpigmented parasites of the red blood corpuscles of reptiles and amphibians that could not be included with other intraerythrocytic sporozoans (*e g Haemogregarina*) may be representatives of mammalian piroplasmata in cold blooded animals Thus the genus *Dactylosoma* is usually placed with genera *incertae sedis* but is considered to be closely related to *Babesia* Noller (1913) believed the genus to be intermediate between *Plasmodium* and *Babesia*

Seven species of *Dactylosoma* (*D ranarum D tritonis D amaniae D jahni D mariae D sahelini* and *D sylvatica*) have thus far been recorded The type genus *Dactylosoma* Labbe (1894) is characterized by relatively large trophozoites which by typical schizogony produce 4 to 16 merozoites When nuclear multiplication is complete merozoites are formed by the development of buds on one side of the parasite only producing a structure that has a hand like appearance hence the name of the genus When the number of merozoites is four which is not typical the resemblance to *Babesia* is striking

This method of schizogony and the varied number of merozoites produced is a regular feature in the type species *Dactylosoma ranarum* and in *D tritonis D sahelini D sylvatica* and possibly in *D amaniae* In *D jahni* and in *D mariae* however asexual reproduction occurs by typical schizogony binary




F 1 F l sch zog y m rocyt
F 2 Typ l sett ryth ocyt
F 3 G m tocyt ( ) f th pl m

*Babesiosoma jahni* (= *Dactylosoma jahni* Nigrelli 1929) in red cells of *Trit us vir desce s* stained with Wright's solution. These photographs of the holotype were made in 1929 but have not been previously published. ×2500

schizogony, however, was observed. This type corresponds to that reported in the present paper for *B. jahni*, i.e., without the residual mass.

*Babesiosoma mariae* differs from *B. jahni* in exhibiting only the one type of schizogony, different dimensions of the various stages, different host species

ing insects were investigated as possible intermediate hosts but no positive evidence was found

Both *D salamandrae* and *D sylvatica* differ from *D ranarum* in host and geographical distribution in the size and shape of various asexual stages and in the number (eight) of merozoites produced

*Morphology and Life History of* Babesiosoma *gen nov*

The members of the genus *Babesiosoma* can be readily distinguished from those of the genus *Dactylosoma* by the following features (1) a less granular and more vacuolated cytoplasm in all stages (2) a *Babesia* like nucleus, without a definite karyosome (3) reproduction by typical schizogony binary fission or budding and (4) development of not more than four merozoites, usually arranged in a rosette or cross shaped

On these bases two species are now recognized as *Babesiosoma jahni* (= *Dactylosoma jahni* Nigrelli 1929a), from the common newt *Triturus viridescens* of North America, and *B mariae* (= *Dactylosoma mariae* Hoare 1930) from several species of cichlids of the genus *Haplochromis* from Lake Victoria Africa

In *Babesiosoma jahni*, according to Nigrelli (1930) two types of schizogony are encountered in Wright stained material In one type illustrated in FIGURES 1 to 7 the first nuclear division is at right angles to the long axis of the schizont and the daughter nuclei remain at the opposite poles (FIGURE 6) Each of these polar nuclei also divides giving rise to a tetranucleate plasmodium (FIGURES 1 and 7) Cytoplasmic division initiates at the poles and extends only to about the middle third of the body of the trophozoite The daughter organisms are separated around a central axis forming a rosette with or without a residual mass at the center of the formation In the observations reported here this type of merozoite production but without the residual mass was most commonly encountered (FIGURES 2 4 5 and 7)

In the other type of schizogony reported by Nigrelli in 1930 the tetranucleate plasmodium undergoes two successive fissions The first takes place along the major axis and completely divides the trophozoite into two binucleate forms The second fission at right angles to the first divides each of these into two merozoites which finally become arranged in a rosette of four individuals without a residual mass Stages interpreted as gametocytes were found (FIGURE 3) and it was suggested (Nigrelli 1930) that the forms with small nuclei were the male gametocytes while those with large nuclei were the female gametocytes

The complete cycle has not been established for either species of *Babesiosoma* although sporozoitelike forms of *B jahni* were observed in the plasma of the newt (Nigrelli 1930) Parasitic leeches were suspected as the transmitting agents although it is possible that mites may be involved the transmission occurring in the land stage of *Triturus viridescens*

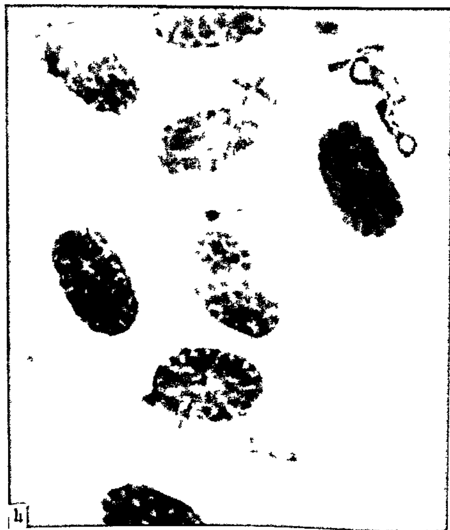
*Babesiosoma mariae* (Hoare 1930) was found in the erythrocytes of *Haplochromis nubilis H cinereus H serranus* and *H* sp members of the family Cichlidae from Victoria Nyanza near Entebbe Uganda East Africa All stages of the parasite basically resembled those of *B jahni* Only one type of




F 5 *Bb so m j k* M lt pl fect in leat 1 ed ll ryth pla t d pa type t k f m wt posed t \ radiat ×1800

single isolated and often light infections although the parasites have been described in frogs and toads from many localities (TABLE 1) Laveran (1898) observed the infection in many European frogs (*Rana esculenta*) and found the disease more frequently in animals examined in summer than in winter Dutton Todd and Tobey (1907) claimed to have seen drepanidia in almost every African frog and toad examined the species included *Rana galemensis R oxyrhynchus R mascarensis Rappia marmorata* and *Bufo regularis* There is no way however to determine whether or not all the forms seen were actually drepanidia *i e Dactylosoma ranarum* The above authors also noted the association of these intracellular parasites with trypanosomes a fact reported by several investigators and emphasized by Billet (1904) who believed that these parasites represented stages in the life cycle of trypanosomes

The rarity of these cold blooded haemosporidians is also indicated for other species Thus Fantham Porter and Richardson (1942) in their Canadian studies found only one fish (*Salvelinus fontinalis*) infected with *Dactylosoma*




Fi E 4 *B b w m j k* V t g th d l pm t f p typ tak f m wt p d
t V radiat V lat pp t th f rm × 100

and different geographical distribution Although stages that were interpreted as young and mature male and female gametocytes were recognized by Hoare no further information was obtained for the missing stages (*e g* sporogony) in the life cycle of *B mariae*

*Incidence of* Dactylosoma *and* Babesiosoma *Infections*

Dactylosomid infections appear to be asymptomatic and because of this the parasites may actually be more common than reported in the literature The reports by the majority of investigators on *Dactylosoma ranarum* deal with

TABLE 2

PERCENTAGE INFECTION WITH BABESIOSOMA JAHNI IN THE BLOOD CELLS OF ONE ADULT TRITURUS VIRIDESCENS

| Cell type | % non infected | % infected |
|---|---|---|
| Erythrocytes | 65.5 | 6.5 |
| Erythroblasts | 0.6 | 0.8 |
| Microcytes | 1.2 | 1.0 |
| Erythroplastids | 0 | 0.4 |
| Pseudoerythroplastids | 0.2 | 0.0 |
| Cells without hemoglobin | 1 | — |
| Degenerated cell | 1.4 | — |

plastid, an element in which the cytoplasmic portion is reduced or almost absent (Nigrelli 1929b; Jordan 1938; Jakowska and Nigrelli 1950, 1952).

The dactylosomids are found in the cytoplasm of the erythrocytes and erythroplastids. They do not, as a rule, cause the cells to rupture, probably because of their small size. Displaced or deformed nuclei of the erythrocytes, swollen cells or dehemoglobinization have been reported (Kruse 1890; Dutton, Todd and Tobey 1907; Noller 1913; Fantham, Porter and Richardson 1942). We are in agreement with some of the other early investigators (*e.g.*, Laveran 1898; Mathis and Leger 1912) that infection with dactylosomid produces very little or no pathological manifestations.

Nigrelli (1930) stated that the blood of newts infected with both *Babesiosoma jahni* (= *Dactylosoma jahni*) and *Trypanosoma diemyctyli* presented a high percentage of abnormal erythrocytes and erythroplastid. He remarked, however, that these degenerative changes were never found in the cells infected with *Babesiosoma*. In the present studies, a mild pseudoerythroblastic anemia and trypanosomiasis (FIGURE 6) were associated with the infection. The one heavily infected irradiated newt reported here (TABLES 2, 3, and 4) showed no changes in the peripheral blood that could be attributed to either parasites or to radiation (TABLE 2), although the activity of the granulocytopoietic layer of the liver and erythropoiesis in the spleen were somewhat depressed.

TABLE 3

PERCENTAGE OF CELLS IN ONE ADULT TRITURUS VIRIDESCENS SHOWING SINGLE AND MULTIPLE INFECTIONS WITH BABESIOSOMA JAHNI

| | |
|---|---|
| single | 80.5% |
| double | 17.0 |
| triple | 2.0 |
| quadruple | 0.4 |
| higher | 0.1 |

TABLE 4

PERCENTAGE OF VARIOUS STAGES OF BABESIOSOMA JAHNI IN INFECTED CELLS OF TRITURUS VIRIDESCENS

| | |
|---|---|
| Schizonts (& gametocytes) | 72.0% |
| Plasmodia | 7.3 |
| Rosette | 11 |
| Single merozoites | 9.5 |

TABLE 1

FAMILY DACTYLOSOMIDAE JAKOWSKA AND NIGRELLI 1955 SUPERFAMILY BABESIOIDEA POISSON 1953

| Parasite | Host | Geographical distribution | Author |
|---|---|---|---|
| Genus *Dactylosoma* | | | |
| *D. ranarum* | *Rana esculenta*, *R. temporaria*, *Triton* sp., frogs | Europe<br>Europe | Lankester 1871, 1882<br>Kruse 1890<br>Labbé 1894<br>Laveran 1898<br>Ziemann 1898<br>Sambon & Low 1901<br>Hintze 1902 |
| | toad | Brazil | Durham 1902 |
| | frogs | Tunis, N. Africa | Billet 1904 |
| | *Kippia marmorata*, *Rana gallemensis*, *R. oxyrhynchus*, *R. mascareniensis*, *Bufo regularis* | Cambia, Africa | Dutton, Todd & Tobey 1907 |
| | frogs | Caucasus<br>Europe | Finkelstein 1908<br>França 1908 |
| | *Rana guntheri* | Tonkin, Indochina | Mathis & Léger 1912 |
| | *Rana esculenta* | Europe | Noller 1913 |
| | *Bufo regularis* | Transvaal, S. Africa | Fantham, Porter & Richardson 1942 |
| *D. tritonis* | *Triton cristatus* | Europe | Fantham 1905 |
| *D. amaniae* | *Chameleon fischeri* | W. Africa | Awerinzew 1914 |
| *D. salvelini* | *Salvelinus fontinalis* | Eastern Canada | Fantham, Porter & Richardson 1942 |
| *D. sylvatica* | *Rana sylvatica* | Eastern Canada | Fantham, Porter & Richardson 1942 |
| Genus *Babesiosoma* gen. nov. | | | |
| *B. jahni* (= *D. jahni*) | *Triturus viridescens* | Pennsylvania, U.S.A.<br>Illinois, N. Carolina, U.S.A. | Nigrelli 1929, 1930<br>Jakowska & Nigrelli 1955 |
| *B. mariae* (= *D. mariae*) | *Haplochromis* spp. | Lake Victoria, Africa | Hoare 1930 |

W[illegible] (1946, 1947, 1948, 1949 and 1950) [illegible] report *Dactylosoma* [illegible] several spec[illegible] New [illegible] record[illegible] *B*[illegible] from C[illegible] Am[illegible] and South Am[illegible] *R*[illegible] from Af[illegible] *R*[illegible] from N[illegible] th Afr[illegible] *R*[illegible] B[illegible] Ceylo[illegible]

immature cells that occur in varied numbers in normal circulation. The erythroplastids are the nonnucleated hemoglobin bearing elements that originate by cytoplasmic cleavage of the erythrocytes; a nucleated portion with a small cytoplasmic rim is left and is known as a microcyte. In fish and in amphibians these nonnucleated elements occur normally in small numbers, but they may increase excessively under certain conditions. Hemoglobin deficiency and cell deficiency anemias are known in cold blooded vertebrates. *Triturus viridescens* is subject to a peculiar form of anemia of still unknown etiology, in which the hemoglobin bearing part of the erythrocyte becomes filled by an enlarged nucleus. The final product of the erythrocyte transformation is a pseudoerythro

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| Cell type | % non-infected | % infected |
|---|---|---|
| Erythrocytes | 6[illegible] 5 | 26 5 |
| Erythroblasts | 0 [illegible] | 0 8 |
| Microcytes | 1 [illegible] | 1 0 |
| Erythroplastids | 0 [illegible] | 0 4 |
| Pseudoerythroplastids | 0 2 | 0 05 |
| Cells without hemoglobin | 1 5 | — |
| Degenerated cells | 1 4 | — |

plastid an element in which the cytoplasmic portion is reduced or almost absent (Nigrelli 1929b Jordan 1938 Jakowska and Nigrelli 1950 1952)

The dactylosomid are found in the cytoplasm of the erythrocytes and erythroplastids They do not as a rule cause the cells to rupture probably because of their small size Displaced or deformed nuclei of the erythrocytes swollen cells or dehemoglobinization have been reported (Kruse 1890 Dutton Todd and Tobey 1907 Noller 1913 Fantham Porter and Richardson 1942) We are in agreement with some of the other early investigators (e g Laveran 1898 Mathis and Leger 1912) that infections with dactylosomid produce very little or no pathological manifestations

Nigrelli (1930) stated that the blood of newts infected with both *Babesiosoma jahni* (= *Dactylosoma jahni*) and *Trypanosoma diemyctyli* presented a high percentage of abnormal erythrocytes and erythroplastid He remarked however that these degenerative changes were never found in the cells infected with *Babesiosoma* In the present studies a mild pseudoerythroblastic anemia and trypanosomiasis (FIGURE 6) were associated with the infection The one heavily infected irradiated newt reported here (TABLES 2 3 and 4) showed no changes in the peripheral blood that could be attributed to either parasites or to radiation (TABLE 2) although the activity of the granulocytopoietic layer of the liver and erythropoiesis in the spleen were somewhat depressed

TABLE 3

PERCENTAGE OF CELLS IN ONE ADULT TRITURUS VIRIDESCENS SHOWING SINGLE AND MULTIPLE INFECTIONS WITH BABESIOSOMA JAHNI

| | |
|---|---|
| single | [illegible] 5% |
| double | 1[illegible] 0 |
| triple | [illegible] 0 |
| quadruple | 0 4 |
| higher | 0 1 |

TABLE 4

PERCENTAGE OF VARIOUS STAGES OF BABESIOSOMA JAHNI IN INFECTED CELLS OF TRITURUS VIRIDESCENS

| | |
|---|---|
| Schizonts (& gametocytes) | [illegible]2 0% |
| Plasmodia | [illegible] 3 |
| Rosettes | 11 2 |
| Single merozoites | 9 [illegible] |

Typically the various stages of *Babesiosoma jahni* were seen in the erythrocytes erythroplastids, microcytes some younger red blood cells and in a few of the pseudoerythroplastids The number of infected cells appeared high (S53 out of 1944 blood cells counted) Almost 41 5 per cent of all hemoglobin containing cells however were infected (TABLE 2) and about one fifth of these carried multiple infections Erythrocytes and erythroplastids in particular with double triple and quadruple infections (TABLE 3) were frequently seen and the schizont (and gametocyte?) stages were observed most often (TABLE 4) No *Babesiosoma* was seen in sections of spleen liver, and other organs stained with standard histologic and hematologic methods The number of destroyed cells in smears (smudges) was negligible for such a heavy infection Free parasites were found in the vicinity of the latter, and it is quite probable that these were released from the cells during smearing The pseudoerythroblastic anemia in this animal was not severe and *Babesiosoma* infection of early pseudoerythroplastids was rare (TABLE 2) It is possible that aside from the morphologic transformation in these cells the hemoglobin is basically altered in this form of anemia Such cells probably do not present the proper environment for *B jahni* In the case of *B mariae* no obvious pathology was noted in the infected fish blood cell

### *Taxonomic Position of* Dactylosoma *and* Babesiosoma *gen no*

The exact taxonomic position of *Dactylosoma* has had considerable discussion in the early literature This parasite together with a hemoflagellate was discovered by Lankester in 1871 in the erythrocytes of one frog Lankester (188?) named the parasite *Drepanidium ranarum* and recognized it as a member of the Sporozoa In his second paper (1882) however he believed that the parasite was a coccidian and that certain spores found by Lieberkuhn in 1854 in the kidney of the frog were part of the cycle This interpretation led Hintze (1902) to believe that the form described by Chaus at (1850) as *Anguillula minima* was the same as Lankester s *Drepanidium ranarum* and should therefore have received priority As was later shown *Anguillula minima* is a coccidian now recognized as *Lankesterella minima* It was Lankester s discovery rather than Chaussat s that attracted attention and he was therefore given credit for stimulating research on Haemosporidia

*Drepanidium ranarum* was rediscovered by Kruse (1890) who placed it in the genus *Haemogregarina* It was again reported as *Haemogregarina ranarum* by Celli and Sanfelice (1891) and as *Laverania ranarum* by Grassi and Feletti (1892) who were impressed by its similarity to Haemogregarines and to the human malarial parasite which at the time was called *Laverania malariae* Labbe (1894) reported *Dactylosoma splendens* *Drepanidium monilis* and *Drepanidium princeps* from the blood of European frogs In 1899 he relegated *Drepanidium ranarum* of Lankester to *Lankesterella ranarum* It is now recognized however that *Drepanidium monilis* in part and *Drepanidium princeps* are coccidians and belong to the genus *Lankesterella* Labbe *Dactylosoma splendens* is synonymous with *Drepanidium ranarum* Lankester but since the name *Drepanidium* Ehrenberg (1861) was preoccupied by another protozoan the name *Dactylosoma* Labbe stands the specific name *splendens* is reduced to

synonymy. Hence the type species should read *Dactylosoma ranarum* (Lankester 1882) Labbé 1894 and not *Dactylosoma ranarum* (Kruse 1890) as reported in the literature (Noller 1913 and later authors).

Noller (1913) discussed the validity of all the generic names given for the sporozoan described by Lankester as *Drepanidium*, namely *Lankesterella*, *Haemogregarina*, *Laverania*, and *Dactylosoma*. As they are known today, *Lankesterella* and *Haemogregarina* are definitely Coccidia. *Lankesterella* belongs to the suborder Eimeridea, whereas *Haemogregarina* is included in the suborder Adeleidea. *Laverania* and *Dactylosoma* are Haemosporidia, the former, which is synonymous with *Plasmodium*, is characterized by the presence of pigment granules, the latter by their absence.

Since species of both *Haemogregarina* and *Lankesterella* also occur in erythrocytes of cold-blooded vertebrates, they are often confused with dactylosomids. The fact that the sporogenic cycle has not been found for *Dactylosoma* makes this form somewhat difficult to distinguish from other sporozoans occurring in the erythrocytes. Certain features of the trophozoites (schizonts), schizogony, merogony, and gametocyte development are characteristic and may be used to identify the various forms found in the blood (TABLE 5). Thus, in *Lankesterella*, schizogony and sporogony occur in the endothelial cells of the vertebrate host, and it is the sporozoite that enters the red blood cell to be picked up by the leech, which acts as a transmitting agent. In *Haemogregarina*, sporogony occurs in the gut of the leech, while schizogony, merogony, and gametocyte development occur in the red cells of the vertebrate.

Some confusion could also exist between reptilian dactylosomid and other erythrocyte-inhabiting sporozoans such as *Schellackia*, *Karyolysus*, and *Haemoproteus*. *Schellackia* is an eimerid coccidian closely allied to *Lankesterella*, but differs in that the schizogony and sporogony occur in epithelial cells of the gut of the vertebrate host, with the sporozoites entering the red blood cell. *Karyolysus*, like *Haemogregarina*, is a blood-inhabiting adeleid coccidian in which sporogony occurs in the gut of mites, schizogony in the endothelial cells of the vertebrate host (usually lizards), the merozoites entering the erythrocyte as mature gametocytes.

In addition, there may be some confusion between *Dactylosoma*, *Babesiosoma*, *Haemoproteus*, and certain species of *Plasmodium*. *Haemoproteus* is a pigment

TABLE 5
STAGES OF BLOOD-INHABITING SPOROZOA OCCURRING IN THE ERYTHROCYTE OF COLD-BLOODED VERTEBRATES

| Genus | plasmodium | schizogony | gametocytes | sporozoites |
|---|---|---|---|---|
| *Dactylosoma* | | | | — |
| *Babesiosoma* | | | | — |
| *Lankesterella* | — | — | — | |
| *Haemogregarina* | | | | — |
| *Schellackia* | — | — | — | |
| *Karyolysus* | — | — | | — |
| *Haemoproteus* | — | — | | — |
| *Plasmodium* | | | | — |
| *Pirhemocyton* | (?) | ? | ? | ? |

producing haemosporidian of birds reptiles and amphibians It differs from the typical members of *Plasmodium* in that schizogony occurs in the endothelial cells of the vertebrate host and the merozoites enter the red cells where they mature into gametocytes Sexual reproduction occurs in blood sucking invertebrates The presence of pigment and lack of schizogony in the erythrocytes immediately separates the members of the *Haemoproteus* from *Dactylosoma* and *Babesiosoma*

A number of species of *Plasmodium* have been described from amphibians (Fantham Porter and Richardson 1942) and from reptiles (Fantham and Porter 1950 Poisson 1953a) The forms described as *Plasmodium minasense* Carini and Rudolph 1912 and *P carinii* Leger and Mouzels 1917 *P rhadinurum* Thompson and Huff 1944a and b and *P floridense* Thompson and Huff 1944a and b are of interest because they produce a small number of merozoites Both *P minasense* and *P rhadinurum* show the rosette formation of four merozoites Wenyon (1926) was impressed by the mode of production of only four merozoites in *P minasense* which could not be distinguished from such forms as *Babesia quadrigemina* and *Achromaticus esperuginis* were it not for the presence of pigment In his opinion forms such as *Plasmodium minasense* appear to be the connecting link between the Plasmodiidae and the Piroplasmidae According to Poisson (1953a) *P catesbiana* and *P bufonis* in the red cells of amphibians are in all probability *Haemogregarina* an interpretation in which we concur

In summary *Dactylosoma Babesiosoma Haemogregarina* and *Plasmodium* are the only forms of blood inhabiting sporozoans in poikilothermic vertebrates in which the asexual cycle and gametocyte development is completed in the erythrocytes *Plasmodium* is characterized by the presence of intercellular haemozoin *Haemogregarina* is distinguished from *Dactylosoma* and *Babesiosoma* in that its schizont meront and gametocyte have densely staining cytoplasm and nuclei The plasmodium and the individuals resulting from schizogony are comparatively larger filling the entire blood cell with considerable displacement of the nucleus of the host cell In *Dactylosoma* and *Babesiosoma* as compared with *Haemogregarina* the schizont meronts and gametocytes have scanty weakly staining cytoplasm and nuclei The plasmodium and merozoites are comparatively smaller rarely filling the space between the nucleus and the periphery of the cells even though a single cell may often show multiple infections by various developmental stages of the parasite

The distinction between blood inhabiting Coccidia and Haemosporidia is made on the basis of the motile and nonmotile zygote and the absence or presence of an envelope in which the sporozoites are formed There is some evidence that in *Babesiosoma jahni* the sporozoites are naked (Nigrelli 1930) this would place the members of this genus in the Haemosporidia The rest of the cycle as in many of the Babesiidae is not completely known The members of the Babesiidae are characterized in the circulating blood cells of mammals by the presence of two to four merozoites with scanty and weakly staining (with Romanowsky s stain) cytoplasm and nuclei and lack of pigment These features also occur in certain dactylosomids of cold blooded vertebrates Such dactylosomids also show in certain stages of merozoite production a rosette

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formation strikingly similar to that seen in the red cells of mammals infected with *Babesia quadrigemina B mulans B equi* (*Nuttallia equi*) *Achromaticus vesperuginis* and *Theileria parva* Like the true Babesioids these dactylosomids often produce pear shaped or vermicular trophozoites several of which may be found in a single blood cell

The members of the genus *Dactylosoma* in the opinion of many authors do not rate a position of taxonomic importance being relegated to the genera of uncertain status (Wenyon 1926 Reichenow 1928 Kudo 1946 Hall 1953 Poisson 1953c) There is a growing feeling however that they are related to Babesiidae of mammals Wenyon in 1926 ventured the opinion that certain unpigmented parasites of the red blood corpuscles of reptiles and amphibians which cannot be included with the haemogregarines are possibly representatives in cold blooded vertebrates of piroplasmata (babesias) of mammals Poisson (1953c) supported this view applying it to the genus *Dactylosoma* in particular

Poisson (1953b) created the superfamily Babesioidea for the families Babesiidae (= Piroplasmidae) Theileriidae and Anaplasmidae The family Dactylosomidae was proposed by Jakowska and Nigrelli (1955) as a fourth member of this superfamily to include the nonpigmented haemosporidians of cold blooded vertebrates in which schizogony with the production of 4 to 16 merozoites and gametocyte development takes place in circulating red blood cells The development of gametocytes in the vertebrate host distinguishes the Dactylosomidae from the other members of the superfamily Babesioidea

Two genera *Dactylosoma* and *Babesiosoma* gen nov are included in the family Dactylosomidae with the following species (TABLE 1) *Dactylosoma ranarum* (Lankester 1882) *D tritonis* (Fantham 1905) *D amaniae* (Awerinzew 1914) *D salvelini* (Fantham Porter and Richardson 1942) and *D sylvatica* (Fantham Porter and Richardson 1942) *Babesiosoma jahni* (Nigrelli 1929a) and *B mariae* (Hoare 1930) Both *D tritonis* and *D amaniae* were originally described as *Lankesterella* but were renamed by Wenyon in 1926 Poisson (1953c) is of the opinion that this interpretation is at least partly correct

A critical examination of other intraerythrocytic sporozoans of poikilotherms may reveal forms described under different generic and species names which may fall into the family Dactylosomidae Members of the nonpigment producing genus *Pirhemocyton* (Chatton and Blanc 1914 1916 Brumpt and Lavier 1935 Wood 1935) in the blood of lizards may be dactylosomids or closely related forms

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# THE MANIFESTATIONS AND DIAGNOSIS OF *BABESIA* INFECTIONS

*By* W D Malherbe

*Department of Veterinary Medicine Faculty of Veterinary Science University of Pretoria Onderstepoort Union of South Africa*

ABSTRACT

In spite of a considerable literature on the *Babesia* infections of animals there are comparatively few references to atypical manifestations of the disease The existing references are reviewed in this paper They include cases manifested by ocular respiratory digestive nervous and rheumatic symptoms

Since the causal agents are not always demonstrable in smears of the peripheral blood and since the disease picture can be so very variable the criteria of diagnosis are laid down and discussed A number of cases studied at the Onderstepoort Veterinary Institute Onderstepoort Union of South Africa are described according to the body systems mainly involved Respiratory symptoms include a range from simple catarrhs to intense dyspnea simulating pneumonia A striking group is made up by the involvement of the circulatory system presenting such symptoms as severe ascites asymmetrical edemas and purpuric lesions In the nervous system *Babesia* infections are represented in animals by the manifestation of epileptiform fits aberrations of the gait abnormal actions and postures and peripheral nerve palsies Enteritis and gastritis indicate involvement of the digestive system

The pathogenesis of the atypical lesions is discussed in considerable detail in the light of the pathological and clinicopathological changes that take place in the babesioses These changes include blocking of the capillaries by agglomerations of parasitized red blood corpuscles with consequent local tissue anoxia and intoxication anemia hypoalbuminemia degeneration of the liver and kidneys capillary fragility and marked coagulation defects of the blood

In all these changes and manifestations there is a close parallel with those encountered in malaria from time to time particularly in the malignant tertian form Important references from the literature of malaria are cited in support of this observation

## INTRODUCTION

In a discussion of the manifestations and diagnosis of *Babesia* infections it is expedient to use the dog as the type animal because of the very great wealth of clinical material at our disposal in South Africa Almost every dog over the greater part of the country acquires the infection in some form sooner or later

I propose to limit my discussion to the particular babesioses we have in my country namely infection with *B canis* in dogs *B bigemina* and *B bovis* in cattle *B equi* and *B caballi* in horses *B felis* in cats and *B trautmanni* in pigs The pathogenesis of the disease is so similar in the various species that the deviations from normality in the dog can be applied *mutatis mutandis* for

practical purposes to those in the other animals There is a common symptomatology which in the majority of cases follows more or less the same pattern exemplified in the dog by fever (102 to 105 F ) malaise and listlessness mental depression disinclination to move about anorexia or an unusual fussiness about certain items of food The mucosae progressively lose their color till they become as pale as ivory Icterus develops in advanced or neglected cases and hemoglobinuria is usually a sign of peracute loss of red blood corpuscles which occurs in some cases The spleen is enlarged and is usually found to be palpable if it is at all possible to examine the organ The feces are strikingly yellow in all but very early or peracute cases and the urine is generally well loaded with bilirubin Debility is progressive and if the dog lives long enough emaciation may be extreme As a rule however the animal dies from organic failure before this stage is reached In the majority of cases the causal parasites are readily demonstrated in smears made from the peripheral blood In chronic infections the temperature rise may be irregular the appetite capricious and the loss of condition a marked feature of the illness

The symptoms in the other susceptible domestic animals are essentially similar

Anybody with extensive experience of these diseases however is forcibly struck by the deviate and protean manifestations of the disease picture as it is encountered from time to time There is almost no guise under which the disease does not masquerade at some time or another and it is therefore no accident that the majority of South African veterinarians have a pronounced attachment to their microscopes

One feels considerable sympathy with Nandi (1949) who prefaces a discussion on the unusual manifestations of malaria by saying Malaria is an arch simulator and a great mimic of all diseases The multiplicity and duplicity of the disease cannot be overemphasized

LITERATURE

The babesioses have been recognized as such for little more than half a century and a considerable volume of literature has been built up over this period with surprisingly enough only few references to atypical symptomatology French workers were prominent in the early days in this field In a short review of atypical forms in 1927 Cuille and Darraspen quoted Nocard (1902) as remarking that it was frequently difficult or even impossible to make a diagnosis of babesiosis from blood smears of dogs even on several successive days and that he could confirm the existence of *Babesia* only by means of subinoculation into susceptible pups

Tarant in 1905 described an acute case with very severe nervous symptoms The animal succumbed within 24 hours with agonal emission of dark brown urine Under the microscope the presence of *Babesia canis* was confirmed

Cadeac (1910) ascribed an outbreak of severe ulcerative keratitis in dogs used for hunting in a swamp in France to babesiosis but this opinion lacked the support of smear examination In 1925 Cauchemez described an outbreak of babesiosis in the clinical forms of divers paralyses of aphasia and of pulmonary forms but Cuille and Darraspen (1927) in quoting his paper do not

mention whether the diagnosis was confirmed by microscopic detection of the parasites This lack of any statement about confirmation applies also to the observations of Cheron (1925) His cases of babesiosis were completely lacking in mucosal pallor hemoglobinuria, or notable alterations in the appearance of urine

The paper of Cuille and Darraspen (1927) gives the best account available to me of atypical forms as observed in France Their diagnostic criteria appear to be entirely satisfactory and their observations covered a wide range Their atypical forms were seen in the guise of respiratory digestive, nervous and muscular aberrations which by their intensity masked the usual symptoms of *B canis* infection

In their study the *respiratory manifestations* affected the upper respiratory tract, the bronchi and the lungs One case showed a slight rise of temperature and a hard painful cough productive of a mucous or mucopurulent discharge The laryngeal region was painful on palpation and the picture was one of an ordinary laryngotracheobronchitis until yellow discoloration of the urine led to suspicion of babesiosis Smear examination showed an infection of *B canis* Other cases showed pulmonary edema with dyspnea dry cough with fluid and even bloodstreaked discharge In cases showing severe symptoms of bronchopneumonia the temperature was elevated, respirations were dyspneic and accelerated and painful paroxysms of coughing were observed Detection of anemia and of bile pigments in the urine led to blood smear examination which gave a positive diagnosis

The *gastrointestinal forms* were as confusing as were the respiratory Some animals showed a rise of temperature and severe obstipation resistant to both purgatives and enemas Other cases appeared in the guise of persistent vomiting with severe thirst anorexia constipation or diarrhea and some abdominal pain In all these the diagnosis of babesiosis was arrived at by smear examination after the changes in mucous membranes and urine had been noted

Babesiosis was also found to be accompanied by *nervous* symptoms beginning with some incoordination of the hind legs and followed a few hours later by a more or less complete paraplegia

Rheumatic manifestations such as great tenderness in all the muscular masses particularly in the region of the back and loins were described The slightest touch produced plaintive cries and the animals moved with great difficulty The usual treatment for rheumatism failed but specific treatment for babesiosis brought about an immediate response

Cuille and Darraspen moreover regarded *chronic babesiosis* as atypical for the reason that it was so frequently difficult to diagnose There were more or less depression a variable and capricious appetite progressive exhaustion and a loss of condition the mucosal pallor alone giving a clue to the real disease The most meticulous smear examination repeated over some days was necessary to find the parasites always rare in number

Lesions of the eyes resembling those occurring in other protozoan diseases such as toxoplasmosis trypanosomiasis and leishmaniosis of dogs were also found Lavier and Lombeure (1922) found in a systematic examination of a dog that had died of acute babesiosis that though the peripheral blood con

tained few parasites the capillaries of the retina and ciliary body were positively bulging with parasitized cells Other cases of theirs showed transitory keratitis and iritis and in one there was a large triangular opaque body in the aqueous humor of the left eye In this latter instance the iris and cornea were not affected

Of South African authors Parkin (1931) described a very acute case of biliary fever characterized by ulcerative stomatitis Recovery occurred completely within two or three days after specific treatment Belonje (1944) described cases showing gastritis with vomiting and enteritis that responded equally well A good case of cerebral' babesiosis has been encountered by Trean (1950) When presented for examination a dog an eight month old collie showed a temperature of 106 F and a heavy infection of *B canis* in the blood Illness had developed suddenly A subcutaneous injection of quinuronium sulphate was given but after a few hours nervous symptoms resembling those of hysteria developed The dog tended to push his head under chairs or other objects and whined incessantly Control over the hind quarters was soon lost the animal trying to get up by raising the fore quarters with the head thrown back Repeated peroral doses of three grains of pentobarbitone (15 grains in all) were required to quiet him for a period of about two hours After each such period however the symptoms returned Finally the animal was treated with phenamidine and placed under deep anesthesia with intravenous pentobarbitone for 36 hours (30 grains being used during this period) When full consciou ness returned the cerebral symptoms had disappeared and recovery was uneventful

Since an earlier report made by Malherbe and Parkin (1951) Brown (1955) one of our younger veterinarians has collected some useful data while in practice An interesting symptom he describes is *masseteric myalgia* which causes dogs to scream with pain if their heads are touched or their mouths opened Quite frequently he encountered *rheumatoid muscular pains* in various other situations chiefly in the legs so that lameness and even paraplegia were prominent symptoms His *cerebral* cases usually started with excitement and restlessness howling and yapping these manifestations then giving way to intermittent fits paresis and sopor Mentality vision and hearing were usually impaired and incontinence of feces and urine often accompanied these convulsive attacks The temperature varied from normal to about 105° F and whether parasites could be found easily or not bile pigments could always be demonstrated in blood and urine Specific treatment was invariably followed by satisfactory response

Another colleague de Boom (1955) recalls a case he treated that resembled a mild degree of strychnine poisoning This dog had no typical symptoms of babesiosis not even parasites in blood smears but showed polychromasia in the blood Specific treatment directed against presumed babesias was followed by immediate recovery

*Babesiosis of Cattle*

In a recent report Zlotnik (1953) has described cerebral forms in about 15 cattle of all ages in Nyasaland in East Africa The disease in most cases

was of sudden onset a temperature of 106 to 107° F being recorded within a few hours There were grinding of the teeth complete loss of appetite and a staggering gait the cows going down with their heads twisted to one side or standing with their foreheads pressed against a wall Visible mucosae were often injected and occasionally jaundiced Urine was usually not discolored except possibly just before death The post mortem examination was reminiscent of heartwater a rickettsial disease Smears from the peripheral blood and from the spleen only rarely showed *B bigemina* Brain smears showed capillaries with swollen endothelial cells and numerous red blood corpuscles nearly all parasitized with *B bigemina* *Cowdria ruminantium* colonies indicative of heartwater were in no case found in the endothelial cells The response to specific treatment was usually not very good Blood changes were not much in evidence so that the writer concluded that the parasites accumulate and multiply in the cerebral capillaries only and that the symptoms were due mainly to the local mechanical and toxic effects produced by them

*Babesiosis of Horses*

I am indebted to Tarr (1955) for a description of some cases of babesiosis in four to five month old foals during January our midsummer Tarr was called in because the foals were behaving most peculiarly They would place their heads between their forelegs with the frontal surface on the ground as if they were contemplating standing on their heads Now and again they would overbalance They were restless and nervous walked in circles a great deal with considerable incoordination and often pushed against stable walls and against their dams Frequently they would nuzzle the dam as though to suck but never actually took milk The temperature went up to 106° F and the pulse rate to over 80 The mucous membranes were very injected and petechial hemorrhages were present Blood smears showed the presence of *B caballi* not the more usual *B equi* as mostly seen in adult horses Treatment with trypan blue solution intravenously was followed by complete clinical recovery within 12 hours

Babesia trautmanni *Infection in Pigs*

The original diagnosis of babesiosis in pigs by the Russians Kowalewski and Demetjew in 1911 was made in pigs showing febrile reactions associated with gastric disturbance the latter not a typical feature of babesiosis in general Cerruti (1938) in a study of the disease described fever hemoglobinuria jaundice and anemia conforming with the usual picture In a recent report Lawrence and Shone (1955) of Rhodesia described apart from the usual symptoms the frequent occurrence of abortion in sows suffering from the disease In all probability this complication can be ascribed rather to the fever than to any organic changes in the animals These investigators did not observe icterus in any of their cases The demonstration of parasites in smears from the affected animals apparently did not produce any difficulties

*Feline Babesias*

Brown (1955) in his report described fairly normal symptoms notably anemia and icterus but found that temperatures in his few cases were not

elevated At Onderstepoort we have found little more than malaise sometimes with evidence of diarrhea

Earlier reports published by Jackson and Dunning (1937) and by McNeil (1937) described symptomatology conforming closely to the standard pattern

### Atypical Forms Studied at Onderstepoort Criteria of Diagnosis

The mere finding of parasites in the blood of a sick dog does not necessarily mean that all the symptoms shown are due to babesiosis There may be a concurrent disease present or since immunity in this disease is not sterile any acute attack of febrile disease or severe organic disease might provoke an exacerbation of the labile *B canis* infection so that the two diseases may then exist side by side Moreover subinoculation of blood into susceptible pups would provide no proof of active infection as the pups would usually become infected if the patient were in a state of premunition

For practical purposes the atypical cases have to be evaluated on the basis of their response to specific therapy therapy that would have no other effect than to kill babesias This response must be rapid and complete and the animal must recover without any supportive treatment aimed at the particular atypical symptoms present Diagnosis or at least a presumptive diagnosis of *babesia* infection is of course desirable in all these cases The diagnosis is regarded as definite if there are parasites to be found and if specific treatment is followed by a further course of events which from experience we regard as the normal one A presumptive diagnosis is usually made from the presence of *increased* amounts of indirectly reacting bilirubin in the serum van den Bergh test in the absence of trauma or evidence of hemorrhage—in the case of dogs and cattle—of *any* indirect bilirubin From experience too we regard certain features of the clinical and laboratory examination as in a degree suspicious *e g* splenomegaly increased bleeding time anemic blood figures and accelerated erythrocyte sedimentation where these cannot be otherwise explained

The specific drugs referred to above and mostly used are phenamidine the quinuronium sulphate group and occasionally trypan blue

Cuille and Darraspen (1927) tested the cases they described by intravenous injection of trypan blue and they correctly stress the importance of a thorough examination in order if possible to make a diagnosis

In rare cases where specific treatment is applied late the confirmation by rapid response to specific treatment may fail on account of the development of secondary changes *e g* insufficiency of the liver or kidneys or both or the presence of concurrent disease such rickettsiosis or severe worm infestation For the purpose of this study however such cases are not included as they would tend to cloud the issue

### The System Incidence of Cases Studied

For convenience the cases studied at Onderstepoort are described according to the systems with which the main symptoms were found to be associated

*Respiratory System*

Dogs are occasionally presented at the Onderstepoort small animal clinic showing what appears to be pneumonia Dyspnea is a very prominent symptom the pulse is small and weak and there is an elevation of temperature Percussion and auscultation however reveal no evidence of pneumonia On further examination the mucous membranes are found to be pale and blood smear examination reveals the cause of the disease to be babesiosis Anemia, together with myocardial weakness could provide the explanation for the dyspnea

Disease pictures due to *B canis* infection, varying from simple catarrhs to that of typical respiratory distemper have been seen with such frequency that it has long since become the established practice at Onderstepoort in these cases to examine blood smears and in many cases also the urine The lungs may or may not be involved in the inflammatory process but the response to specific therapy is quite striking The symptoms as a rule disappear fairly rapidly without any supportive treatment Obviously if the correct etiology is not recognized and the specific therapy is not employed treatment of these cases would be foredoomed to failure

*Circulatory System*

Rather remarkably in view of the frequency with which they are encountered at Onderstepoort cases of severe circulatory derangements as far as I am aware have never been described in the literature The derangements are shown as edemas of the subcutis accumulations of transudate in the body cavities (notably ascites) and purpuric lesions

*Ascites cases* These present a characteristic picture in which abdominal distention is usually but not invariably coupled with advanced emaciation The subjects are usually half grown pups or young dogs seldom more than one year old In some cases severe subcutaneous edema of the ventral abdominal region often giving the impression of skin transparency accompanies the extreme distention of the peritoneal cavity

On admission these dogs usually have very pale mucous membranes normal or decidedly subnormal temperatures (about 96 to 97 F ) and blood smears may or may not show the presence of parasites On occasion many hours have been spent on examining blood smears from such dogs and not a single parasite has been found at any stage of the examination All these cases in the urine examination show the presence of bilirubin which is found to be derived from the indirect type in the plasma by the van den Bergh diazo test On such strong presumptive evidence these animals are treated specifically for babesiosis They are not subjected to paracentesis however desirable this procedure may seem It has repeatedly been shown that this is unnecessary and in fact paracentesis is regarded as contraindicated on account of the danger of the development of extensive subcutaneous edema from direct escape of fluid through the puncture tract and because of the danger of cardiac collapse The results obtained from specific treatment are striking The temperature rises to the normal range within 24 hours The peritoneal tran







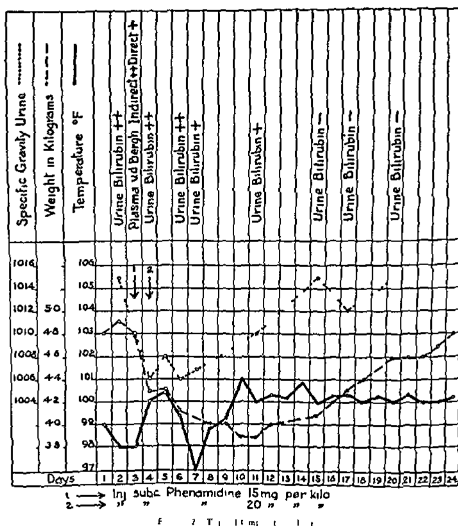
Figs. 1a and 1b. Ascites case before and after treatment.

sudate is rapidly reabsorbed, this process being accompanied by polyuria and low urinary specific gravity. The disappearance of fluid is complete after 7 to 12 days and the specific gravity of the urine returns to a normal figure in the same time. The improvement in clinical appearance, due to decreasing peritoneal transudate, is accompanied by a daily drop in weight. This decrease continues till the process is complete. The weight then remains static for two or three days, after which normal weight increase takes place with the improvement in body condition.

Many of these cases are complicated by more or less severe ancylostomiasis which is not treated until the ascitic fluid has entirely disappeared. The presence of hookworm infestation can therefore be presumed to have no direct bearing on the pathogenesis of ascites. Moreover, a number of cases of ascites have occurred in animals free from worms.

*Other edemas.* Some years ago an outbreak of morbidity and mortality in a group of greyhounds was investigated. The condition was characterized in a number of these animals by edemas of varying distribution. These swellings, however, were mostly associated with the scrotum and had a clinical appearance suggestive of hydrocele. Simultaneously, some of the dogs showed circumscribed edema in other parts, *e.g.* the face and joints. Examination of blood smears showed the presence of *B. canis*. Specific treatment brought about the disappearance of edema in 48 hours, with an improvement already evident in 24 hours.

In recent years edemas in cases of babesiosis have come to be regarded as a not unusual symptom. An interesting observation is that these lesions show no particular tendency to be disposed symmetrically. Edemas have been noted involving one or both ears, a single joint, a line along one mandibular ramus




[illegible]

the periorbital region the lips and the whole head In young pups edema of the periorbital region and of the whole face have also been observed in association with ascites

*Purpura hemorrhagica* Much less frequently than in the ascites cases dogs are encountered that show the clinical picture of purpura To this form too no reference has been found in the literature Hemorrhages varying from pin point petechiae to ecchymotic patches up to 3 or 4 cm in diameter (with irregular outline) are seen involving the [illegible] the iris the buccal or gingival mucous membranes and usually the skin of the abdominal wall on either side of the prepuce and the skin on the inside of the thighs Some of the cases void red urine on occasion containing blood coagula Similar clots have been



[illegible] Edema [illegible] head before [illegible] treatment [illegible]

observed in feces indicating hemorrhage into the posterior bowel and also in the mouth from gingival bleeding

The temperature may or may not be elevated and blood smears may or may not show parasites The course has been found to be very variable from acute disease to ill-defined chronic malaise The response to specific therapy is as satisfactory as in the typical disease Extravasations disappear progressively over a few days the time depending on the extent and degree of hemorrhage and this is accompanied by a return of appetite and improved habitus

One case of this type dog DOB 649 aged two years had been treated for suspected tonsillitis with penicillin and chlortetracycline but had shown no improvement and was off its feed taking only a little milk The owner complained that the dog had been hemorrhaging from the mouth This was still in evidence Examination revealed the tonsils to be of normal size but they resembled blood clots The abdomen was tense and could not satisfactorily be palpated The temperature was normal and blood smears negative

Urine examination showed the following the color an opaque dirty green sp gr 1 044 pH [illegible] albumen +++ Sheftel s test for sugar negative Rothera s nitroprusside test for ketone bodies negative bilirubin +++ The centrifuged deposit consisted of blood elements and amorphous material There was no evidence of inflammatory changes in the kidneys or urinary tract Spectroscopic examination showed the absorption bands of hemoglobin The impression gained was that whole blood had been added to the urine by hemorrhage somewhere in the urinary tract This fact coupled with the hemorrhaging tonsils inspired closer examination of the visible mucous membranes and skin Extensive ecchymoses were found in the skin of the abdomen near the prepuce and the inside of the thighs A diagnosis of purpura was now in order

Two days after admission the two hind legs the right foreleg, and the scrotum were found to be edematous

Blood examination revealed a red cell count of 4 56 million per c mm white cell count, 11 600 per c mm , hematocrit 32 per cent, hemoglobin 9 15 gm per 100 ml , nonprotein nitrogen 49 mg per 100 ml plasma van den Bergh reaction indirect ++ direct trace The plasma examined spectroscopically showed the presence of hemoglobin There were therefore anemia, hemoglobinemia and evidence of a hemolytic process

A presumptive diagnosis of hemorrhagic purpura due to *B canis* infection was then made and the dog was treated by subcutaneous injection of phenamidine (B Vet C ) 0 4 cc of 5 per cent solution per kilo

Urine was examined daily and within a week bile pigments hemoglobin blood and albumen had disappeared and the specific gravity had returned to normal Blood examination showed a slight improvement in the anemia, always the slowest part of convalescence in uncomplicated babesiosis and the van den Bergh test showed only the faintest trace of indirect bilirubin The tonsils now were of a healthy color and normal size while the purpuric lesions had all been absorbed The edema of the legs and scrotum needed but four days after treatment to disappear completely The dog s interest in food revived rapidly and, after a further 10 days' observation the animal was discharged fit

### *Nervous System*

The cases in which the nervous system was involved were representative of lesions of both the central nervous system and the peripheral nerves

Symptomatology indicating involvement of the *central nervous system* was shown in the following cases

(1) A dog while walking showed a peculiar action of the front limbs which were kept extended when moved forwards and were swung rather vigorously from the ground position through a large arc forwards and upwards toward the chin after which they were returned sharply to the ground The effect produced resembled a parade or goose step The blood smears were positive for *B canis* and the only treatment instituted was a specific one for babesiosis The locomotor symptoms disappeared rapidly and the animal subsequently remained clinically healthy

(2) A dachshund was admitted with a history of fits of recent onset These were spaced at short intervals (several being observed during the afternoon of admission) and left the dog exhausted No parasites were found in the blood smears The patient died the same night Histopathological examination of the hippocampus of the brain showed capillaries tightly packed with parasitized erythrocytes

(3) A Dalmatian bitch had been treated for babesiosis on three successive days by her owner The doses of the specific drug used had been inadequate and when the dog was admitted on the fourth day blood smears were still positive In addition to the usual symptom of the disease the animal showed rhythmic backward and forward synchronous movements of both ears at a rate of two per three seconds On the fourth day after adequate treatment the

movements stopped whenever the dog s attention was distracted The next day the movements were less marked and were observed only now and again By the eighth day they had completely disappeared

*Peripheral nerve lesions* were observed in one case that was interesting be cause of the reappearance of the original nerve symptoms when the animal relapsed to babesiosis The dog a 6 5 year-old Rottweiler trained as a police dog had recovered from *B canis* infection six months prior to its admission It then showed weakness and stiffness of the hindquarters Blood smears were positive for babesiosis and specific treatment brought about recovery with disappearance of all symptoms Shortly afterwards it relapsed, and then a striking symptom was the dragging of its right hind leg with the dorsum of the toes in contact with the ground Blood smears were again positive and specific treatment resulted in the disappearance of the leg symptom A second relapse followed with reappearance of the same paralysis in the right hind leg but this time specific treatment was not followed by a further relapse and yet did not bring about disappearance of the gait symptoms After autopsy six months later during which period these symptoms had persisted a histopatho-logical examination of the right sciatic nerve showed evidence of severe degen erative changes

### *Digestive System*

This system in typical babesiosis is affected to some extent in that a vary ing degree of constipation is present in the majority of cases The symptom can therefore not be regarded as atypical Cases of gastritis with vomiting and abdominal pain however are occasionally found to be due to infection with *B canis* One case had been under treatment for a week for gastritis without showing any improvement at all Its condition had in fact deterio-rated progressively On admission to the Onderstepoort Veterinary Labora tory examination of blood smears showed *B canis* to be present and specific therapy was instituted No attempt was made to treat the gastritis yet in 48 hours recovery was complete

In this connection mention should also be made of gastritis and enteritis as a result of uremia supervening as a complication of babesiosis It has fre quently been found that this disease directly affects by intoxication both the kidneys and the liver If a patient already near the border line of kidney decompensation contracts the disease the additional load would readily pro-vide the last straw with consequent renal or hepatic failure

## Discussion

The usual symptomatology produced by the various species of *Babesia* is very similar in the different species of domestic animals It is clear from the evidence here assembled that the aberrant manifestations also fall into a simi lar pattern of forms In a discussion of the pathogenesis of these atypical disease pictures it now appears profitable after considering the mechanisms by which these could be brought about to relate to these the known facts pertaining to babesiosis in order to arrive at conclusions as to which mecha nisms are likely to be operative in the various instances

It is a well known pathological sequence for instance that interference with the blood supply of an organ or any portion of an organ leads to tissue anoxia which will result in modification or loss of function of that organ or portion The degree of loss of function would be determined by the degree of disruption or interference

Knisely and his coworkers (1947) in a series of studies of capillary circulation in normal and diseased patients noted the existence of what they termed the sludging' phenomenon in diseases of widely varying etiology This intravascular agglutination or sludging of blood cells in capillaries however has been observed on many occasions in infections with various protozoa Fairley (1946) *e g* in a textbook description of malaria mentions a number of manifestations classified as acute pernicious malaria which are mainly dependent on internal sporulation and localized blockage of the capillaries by *P falciparum* in different organs such as the brain heart intestines and spleen by parasitized corpuscles which adhere to one another and to the capillary epithelium ' He holds this fact responsible for the diverse symptomatology of malignant tertian malaria

Van Rooyen (1952) quotes other authors including Manson Bahr who states Blocking of the capillaries of the brain by the red blood cells containing parasites is the chief cause of cerebral symptoms Jewel and Kauntze similarly mention sudden blocking of the cerebral vessels by red blood cells containing subtertian parasites and if not recovered from speedily may lead if life is prolonged sufficiently to necrosis of the portion of the brain served by the occluded vessels while Dubois and van den Bergh write in their text book that fatal cases have been reported where cerebral capillaries only contained parasites

Talbot *et al* (1949) demonstrated electroencephalographically the incidence of epileptic (*grand mal* type) seizures in soldiers with tropical types of recurrent malaria Some of these changes were reversible with energetic antimalarial treatment but in some patients the damage was permanent Talbot and his associates concluded that chronic recurrent malaria must be considered in the differential diagnosis of convulsive seizures

Maegraith (1948) is another author who in his studies of pathological processes in malaria and blackwater fever has made similar observations The subjective side of the symptomatology of cerebral malaria is interesting He describes odd behaviour confusion passing into acute delirium with hallucinations accompanied by violence The patient complains of increasing head ache and becomes drowsy or restless and depressed He continues into coma slowly and progressively One can easily imagine the existence of similar feelings in the animal cases

Clark (1918) drew attention to agglomerations of parasitized cells in the capillaries of the brain in cattle affected by *B bigemina* infection in Panama He nearly always found them more easily in preparations from the brain than from elsewhere The drawings in Zlotnik s (1953) article illustrate such agglomerations in brain vessels In canine babesiosis Lavier and Tombeure (1932) found the retinal and ciliary body capillaries to be bulging with cells containing the causative parasite Heavy parasitization of clumped erythro

cytes in brain capillaries in both canine and bovine babesiosis have been observed on a number of occasions by Clark and Parkin (1942) Malherbe (1944) and de Boom (1955) all of Onderstepoort in cases where smears made from peripheral blood of the same animals had shown rare or no parasites In these cases brain derangements had been much in evidence the dogs symptoms taking the form of fits and the clinical features of the cattle resembling those of heartwater a disease usually howing incoordination some loss of equilibrium and pronounced hyperesthesia A case described by Piercy (1947) died quite suddenly without showing any warning symptoms and in this case numerous parasites and hemorrhages were found in the brain Purchase (1947) reported also from East Africa that in five years he had diagnosed three cases of what he called cerebral babesiosis in dogs from pathological sections examined for rabies Another case a dog that he could examine clinically was comatose though still having a temperature of 102 F Parasites were difficult to find in ordinary smears but were numerous in the brain after death They were mostly extracellular filling the lumen of capillaries and small arterioles the distribution in the brain being very uneven

Cuille and Darrasen (1927) also stressed in their review the rôle of what they called parasitic emboli trapped in the capillaries in the symptomatology of atypical babesiosis Brumpt (1919) also referred to parasitic emboli but regarded them in the light of being the main seats of multiplication of the parasites

The views on pathogenesis originally expressed by Malherbe and Parkin in 1951 have received ample support in the pathological studies of Gilles and Maegraith (1954) In their laboratory induced cases the latter investigators did not see actual cases of cerebral babesiosis but in the histological examination of the brain they noted small vessels blocked with massive accumulations of parasitized cells and free parasites They remarked also on the tendency of parasitized cells to layer themselves along the endothelium of the blood vessels as seen in *falciparum* malaria The endothelial cells of the smaller vessels throughout the brain substance frequently showed signs of degenerative change being swollen and occasionally granular and fatty Perivascular escape of erythrocytes leading to macroscopic hemorrhage occurred These authors incriminated the basic disturbance giving rise to stasis or impedance of the capillary flow as one by which fluid escaping through abnormally permeable vessel walls would leave the erythrocytes concentrated and compressed Tissue damage from anoxia and the accumulation of toxic metabolic products resulting from such stasis would thus readily account for the symptomatology that has been described

On this evidence it could be expected that a variety of organs and tissues would show deviations from their normal functioning The relative or absolute anemia of organs resulting from such capillary blocking could quite conceivably open the way to invasion by various viruses and bacteria resulting in symptoms apparently unrelated to those of babesiosis The rapid response to specific babesiosis therapy a treatment known to be ineffective in the secondary conditions would support the incrimination of *B canis* as the important etiological agent

4b

4a

Fı u e 4 d 4b C t l b la l dam g l b b

The whole explanation for edema and ascites in babesiosis cannot be found in any single mechanism or change in the body The most important common factor is the lowering of serum protein level particularly that of serum albumin In human medicine ascites develops frequently in both acute and chronic liver disease Weir (1947) states that portal hypertension long considered as the most important causative factor cannot be demonstrated in all cases and he lists other important influences as lowering of the serum proteins disproportionate decrease in the serum colloid osmotic pressure dietary factors disturbance of the ability of the liver to handle water and altered permeability of the capillaries

*Babesia* infections are responsible in fact for marked parenchymatous involvement of the liver Since the earliest days this has been more than evident from the wealth of clinical and pathological material available at Onderstepoort Gilles Maegraith and Andrews (1953) have sketched the development of the liver damage from the early damming of blood in the sinusoids around the central vein through stages of centrilobular atrophy and degeneration of the hepatic cells to necrosis with disintegration and disappearance of these cells In order of severity the damage extended from the area around the central vein to the midzone and lastly encroached upon the peripheral zone FIGURE 4 illustrates such damage Maegraith (1948) has described strictly comparable lesions in malaria

Among the results of this liver damage there is disturbance of the protein regulating function of the organ There is a large amount of evidence of this from chemical and electrophoretic studies which show a reduction of serum albumin to very low levels (Marrack and Hoch 1949) thus paving the way for the development of edemas FIGURE 5 shows the typical pattern from a case of babesiosis described in an electrophoretic study by Polson and Malherbe (1952) The chemically determined figures in one well-developed case with ascites as the prominent symptom were 3 64 gm per 100 ml for total plasma protein with albumin at 0 84 gm per 100 ml and an albumin globulin ratio of 0 33 compared with normal values of about 6 to 7 3 to 4 5 and over 1 respectively Parasites were demonstrated in this case and specific treatment was followed by disappearance of ascites without any other treatment in less than two weeks with coincident rise in the albumin and hence in the other values

Ascites from portal hypertension and chronic liver disease can and does occur particularly in older dogs as well as it does in humans but it is quite unlikely to play any significant role in babesiotic ascites The main etiological mechanisms operating with the hypoproteinemia appear to be the associated anemia general toxemia produced by the parasites in the blood and a relative myocardial insufficiency

The kidneys too are deeply involved in the clinical pathology of babesiosis In dogs it is frequently observed that if kidneys are near the borderline of decompensation a *Babesia* infection will precipitate complete kidney failure and hence uremia Gilles and Maegraith (1954) concluded from their study of this aspect that there was kidney damage with urinary suppression of the same type as in blackwater and which can thus be classified within the wide group of renal anoxic states




FGURES 4 a d 4b C l l b la l erd m g l b b

seconds Capillary fragility from toxic changes in the endothelial cells has been described by Gilles and Maegraith (1954) For practical purposes we have come to regard nearly all purpuras in dogs as due to *B canis* infection Similar lesions are seen in the other domestic animals

Maegraith (1948) describes what he calls dysenteric pernicious malaria characterized by the passage of frequent stools consisting mainly of blood mucus and epithelial and cellular debris There is tenesmus colicky abdominal pain and tenderness of the abdomen particularly along the line of the colon There may be nausea and vomiting The condition in fact is indistinguishable from acute bacillary dysentery Maegraith s photomicrograph of a section of stomach in a case of *falciparum* malaria shows intense accumulation of parasitized erythrocytes in capillaries

As one thus continues the study of atypical forms of babesiosis in dogs and horses and cattle and then compares with them the published observations of symptomatic aberrations of malaria the similarity of pathology and of clinical pathology is inescapable and it becomes obvious that in spite of the differences in the life cycle of the parasites their effect on the body is capable of exactly similar potentialities

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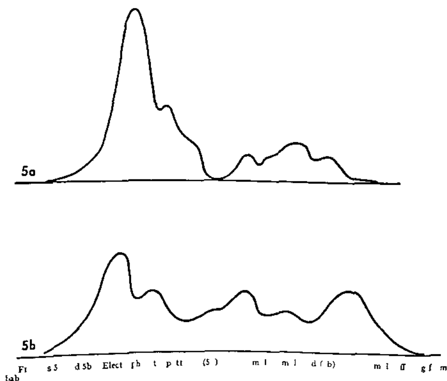
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In animals there is little if any tendency to the formation of the nephrotic type of edema so well known in humans The explanation for the notably asymmetrical edemas in babesiosis must be sought rather in localized stasis or blockage of the small arterioles and capillaries existing with or operating in conjunction with usual causes of edema in disease namely increase in capillary pressure reduction of protein osmotic pressure of the blood and toxic or anoxemic alteration in the permeability of the capillary wall These are all potentially present in babesiosis

In lungs there is the same tendency to dilatation and congestion of the alveolar capillaries Edema is quite a usual terminal event

Purpura or a tendency to hemorrhage has been associated in the past as far as animals are concerned with bacterial infections In malaria however this symptom is well known Shrager and Keen (1946) have described such cases and Fairley (1946) in his systematic description quoted above lists purpuric skin eruptions epistaxis hematemesis hemoptysis melena hematuria and vaginal hemorrhages as symptoms encountered in malaria from time to time Comparable lesions occur in *Babesia* infections where we have the following circumstances favoring the development of purpura in some cases The bleeding time is prolonged Bleeding *Stomoxys* bites on the ears of affected dogs is observed almost daily The one stage prothrombin time test of Quick shows values increased from a normal 11 to 12 seconds to 15 to 20



# TREATMENT AND CONTROL OF BABESIOSIS

*By* J. Carmichael*
*Late Colonial Veterinary Service, Uganda, Africa*

## *Introduction*

The efficient control of any disease depends on a sound knowledge of its natural history, which means a study of its etiology, the source of infection and method of transmission. In the case of babesiosis we know transmission is by ticks belonging to the family Ixodidae. It is essential, therefore, to have a sound knowledge of the ecology and life cycle of the various genera of ticks concerned, for logically control is attained by breaking a link in the etiological chain of the disease. Methods of control in a disease of the blood transmitted by ticks can be directed at the intermediate host and/or at the vertebrate patient which is the ultimate host of the parasite. If the first method is successful the disease is prevented and the necessity for directing measures to the final host does not arise. This desirable state of affairs has been reached in many countries of the world where babesiosis exists.

Treatment and control therefore can be conveniently dealt with under a number of headings, commencing with the disease in the vertebrate host and working backwards to the tick. (1) drug treatment, including specific chemotherapy, symptomatic therapy and clinical care such as nursing and dietetic treatment; (2) premunition, and (3) prevention, including tick control and husbandry.

## *Specific Chemotherapy*

Generally speaking, the *Babesia* have responded better to chemotherapy than most of the Haemosporidia affecting domestic animals and of these the larger *Babesia*, such as *B. canis*, *B. caballi* and *B. bigemina*, in my experience respond to specific drug therapy rather better than the smaller varieties such as *B. gibsoni*, *B. equi* and *B. bovis*. The classical work of Nuttall and Hadwen (1909) led to the discovery of trypan blue, the first effective therapeutic agent discovered. Trypan blue held the field in the treatment of babesiosis for many years. The dye is usually given in a 1 or 2 per cent solution and preferably is injected intravenously. Subcutaneously it may give rise to sterile abcess formation. The tissues are stained blue and recovery is rather slow. Nevertheless trypan blue is still used in the treatment of babesiosis in some parts of the world, especially in *B. canis* infections, and also to some extent in cattle. In this connection it is interesting to recall that Neitz and Steyn (1947) showed that *B. gibsoni* is completely refractory to trypan blue.

The acridine dyes were later introduced by Sergent, Donatien, Lanot and Lestoquard in Algeria, and Trypflavine or euflavine is used quite extensively in equine babesiosis, especially in *B. equi* infections in South Africa. It is administered intravenously in a 2 to 5 per cent concentration and may be combined with quinine hydrobromide in doses of about 5 grams given intramuscu

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The acridine dyes were later introduced by Sergent Donatien Parrot and Lestoquard in Algeria and Trypflavine or euflavine is used quite extensively in equine babesiosis especially in *B equi* infections in South Africa It is administered intravenously in a 2 to 5 per cent concentration and may be combined with quinine hydrobromide in doses of about 5 grams given intramuscu

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larly Trypflavine is also used to some extent in cattle, especially in North Africa for the treatment of babesiosis and theileriasis

More recently the quinolyl preparations were introduced and acaprin was synthetically prepared by Schonhaefer and Henecka and its value as a therapeutic agent for *B canis* was determined by Kikuth (1935) Unlike trypan blue and trypflavine large doses were found to cause not only clinical recovery but also parasitological sterility of the animal On the other hand when smaller doses were administered the recovery was generally associated with retention of the parasites in the system of the animal (Kikuth 1938) and with a state of premunition

I published results of treatment of canine babesiosis carried out in Uganda in 1934 (Carmichael 1935) and pointed out that while acaprin proved to be a very useful drug and superior to trypan blue it was markedly toxic and that the margin between the poisonous and the effective dose was small Often there was quite a severe reaction following the injection of acaprin consisting of motor restlessness salivation vomiting defecation and perhaps collapse These symptoms were sometimes alarming but, as a rule, soon passed off I found that the dose could be repeated after 24 hours with no ill effects but after a month such increased sensitivity seems to develop that an ordinary therapeutic dose might be toxic Nevertheless the quinolyl preparations since then have proved the most popular form of treatment of babesiosis in all animals in every part of the world and they maintain that position today

Lourie and Yorke (1939) investigated the action of the aromatic diamidines in *B canis* infections and found most of the compounds they examined had a definite action against *Babesia* infections in puppies

Adler and Tchernomoretz (1940) working in Palestine found that the diamidino stilbene was effective against *B bigemina* in calves but had no effect against anaplasmal or theilerial infections in doses of 2 mg to 4 mg per kilo

Daubney and Hudson (1941) reported on 16 clinical cases in dogs of *B canis* infections and 2 cases of *B caballi* in horses They came to the conclusion that the diamidino stilbene cured the infections The relapse rate was low but there were a series of reactions following the administration of the drug that were undesirable These reactions consisted of hyperesthesia restlessness swelling of the face and lips and difficulty in breathing In horses the reaction was so severe that it was deemed unsuitable for this animal in spite of the fact that it cleared the babesia infection

Carmichael and Fiennes (1941) reported on 116 unselected cases of *B canis* infections in dogs treated with diamidino diphenoxy propane in a single dose of 5 mg per kilo or 2 5 mg per kilo given subcutaneously on two successive days The results were very satisfactory and there were only 10 relapses and 4 deaths while the drug showed no signs of toxicity at these levels

Later Carmichael (1942) investigated the action of diamidino diphenyl ether in canine babesiosis in 25 unselected cases with completely satisfactory results in that there was 100 per cent cure with no toxic reactions and no relapses. This compound later marketed under the name of phenamidine has been in general use in babesiosis of all animals with mainly satisfactory results As is common with all the diamidines subcutaneous inoculation of concentrated

solutions may prove irritating and occasionally an allergic type of reaction consisting of a transitory swelling of the face and lips may occur but this is not common

The only other aromatic diamidine of importance is the diamidino diphenoxy pentane known as pentamidine or Lomidine This is much favored by the French in North Africa in the treatment of babesiosis in all animals Recently in Great Britain it has been used with success in *B bovis* infections

Finally as far as drug treatment is concerned the recently introduced berenil (Hoechst) should be mentioned This compound is 4 4 diamidino diazoamino benzene diaceturate and is said to be effective against babesia infections of all animals

Summarizing the chemotherapy of babesiosis I think it can be said that the quinolyl preparations hold pride of place at the present time along with the aromatic diamidines and the flavines in that order From the purely experimental point of view *B rodhaini* of mice should be kept in mind as a possible laboratory test organism for future compounds (Beveridge 1953)

As regards further methods of treatment of babesiosis Paludrine has been used with some success in *B gibsoni* infections in Ceylon (Seneviratne 1953) but novarsenobillon given intravenously is probably more efficient (Venkata narusu 1945)

Antibiotics do not appear to have been used to any great extent but Jansen (1953) after experiments with Aureomycin in splenectomized donkeys in South Africa considers that a field trial using 2 5 mg per kilo should be made Jansen found that Aureomycin had a definite parasiticidal effect in *B equi* infections

### *Symptomatic Treatment*

This is secondary to specific therapy but is nevertheless important and good nursing with an adequate diet of a laxative character together with comfortable housing where practicable are essential features in the treatment of babesiosis Details of course will depend on the species of animal and the circumstances of the case In general symptomatic treatment should be directed to improving the anemia and relieving constipation and jaundice but it should be recorded that some veterinarians administer antihistaminics in conjunction with the specific and general treatment and claim good results

### *Premunition*

The nature of the resistance or immunity to the Haemosporidia is difficult to define A sterile immunity rarely occurs except in the case of *Theileria parva* and even the explanation of this phenomenon is open to considerable speculation

In the case of *Babesia* the inoculation of susceptible animals with infected blood sets up a mild or severe attack of the disease depending upon the virulence of the parasite injected Recovery confers upon the animal a resistance or tolerance to subsequent natural infection This condition is associated with the presence of the parasite in the blood and is known as premunition or infection immunity

Advantage is taken of this sequence of reactions for the purpose of artificial premunization and in the present state of our knowledge, this method of conferring resistance to susceptible animals forms a valuable means of protection in the case of importation of cattle into enzootic areas A mild strain of *Babesia* is obtained and a susceptible bovine free from other blood infections such as anaplasmosis is inoculated and is kept under tick free conditions Providing the strain of *Babesia* is suitable this animal goes through a typical temperature reaction recovers and becomes preimmunized Its blood can then be used for preimmunizing other cattle The dose of blood varies from 1 to 10 cc and is given subcutaneously The animals should be inoculated at least three weeks before being exposed to natural infection and should be kept under good conditions and free from ticks Close observation is necessary and the temperature reaction which occurs about the 8th to 10th day after injection should be recorded If necessary symptoms should be controlled by specific therapy At one time cattle were preimmunized in England prior to shipment to enzootic countries but now preimmunizing stations are established at suitable sites to receive imported cattle in the various countries concerned Young stock between 3 to 15 months are the most suitable for preimmunization

Preimmunization of horses with attenuated parasites by passage through donkeys has been attempted (Theiler 1908) but has not been put into regular practice

This type of resistance is not absolute and may last a variable length of time up to about 22 months or perhaps longer (Neitz quoted by Henning 1949) There is evidence however that constant minimal natural reinfection from ticks will reinforce the original artificially produced resistance and thus enable the animals to survive in enzootic areas Breakdowns do occur under special stress conditions such as severe reactions following antirinderpest inoculations and these should be controlled by drug treatment

*Prevention*

*Tick control* The natural transmission of *Babesia* is dependent on the existence of certain species of ticks and infection can therefore be prevented by destroying the ticks or keeping the animal free from tick infestation Species of *Boophilus* are perhaps the commonest vectors of *Babesia* in cattle but many other genera such as *Rhipicephalus* and *Ixodes* are involved A dipping campaign must therefore be planned to suit the particular circumstances and the species of ticks that are implicated This will differ in different countries as will also the acaricidal fluid used and it is impossible here to go into details to suit each area It should be remembered however that resistance to arsenic DDT BHC and other insecticides is not uncommon and plans will have to be formulated in the light of this knowledge

In dogs species of *Haemaphysalis Rhipicephalus* and *Dermacentor* ticks are known transmitters of *Babesia* The animal should be kept free from ticks by the use of the usual hygienic measures such as brushing and grooming and attention to the bedding combined with the use of suitable insecticides usually in powder form

The genera *Rhipicephalus Hyalomma* and *Dermacentor* are all known to

transmit *Babesia* to horses Susceptible horses should be stabled regularly groomed and hand-dressed with suitable insecticides where necessary

In parts of Africa and probably elsewhere an area provided it is adequately fenced can be cleared of ticks by grazing immune indigenous stock that pick up the ticks Regular dipping destroys this infestation and eventually the area becomes tick free and safe for susceptible animals

*Husbandry* As with all disease prevention good husbandry is essential Cattle should not only be dipped but kept in fenced areas so as to avoid reinfection from wild game or other cattle Good housing and proper feeding are necessary to good health and a healthy robust animal will resist disease and respond to treatment better than neglected stock whatever the species may be

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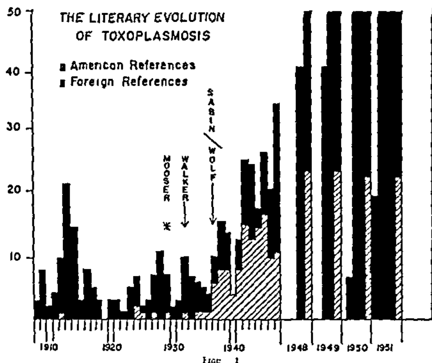



Fig 1

*Reference*

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Part III Toxoplasmosis

# INTRODUCTORY REMARKS

*By* Floyd S Markham

*Lederle Laboratories Division American Cyanamid Company Pearl River, N Y*

Interest in toxoplasmosis has grown remarkably during the past 20 years A glance at the excellent bibliographies in this field prepared by J K Frenkel and Don E Eyles of the United States Public Health Service demonstrates both the cosmopolitan nature of the disease and our increasing recognition of its importance In spite of the fact, however, that *Toxoplasma* were first described in 1908 American workers as a group seem to have been blissfully unaware of these parasites until quite recently When *Toxoplasma* first came to my attention in 1932 only Mooser s 1929 paper gave evidence of their presence in this part of the world

The very bulk of the bibliographies assembled by Frenkel and Eyles prompted me to give a closer look at the distribution of the reports dealing with *Toxoplasma* and the result of this inspection is summarized in FIGURE 1 While it is fairly certain that prior to Mooser s report several American investigators had encountered *Toxoplasma* none recognized and named the parasites as such and most of the American references before 1935 were retrospective diagnoses An exception is the 1932 paper of Walker and Sweeney These workers like Mooser encountered *Toxoplasma* while working with typhus virus in guinea pigs and they propagated and identified the organisms morphologically

It was at about this time that the laboratory study of filterable viruses was rapidly expanding and with it of course the use of experimental animals Study of the encephalitides especially focused the attention of investigators on nervous manifestations in experimental animals Workers began to realize the presence and importance of spontaneous virus infections among laboratory animals Less frequently it was recognized that protozoan infections might easily be mistaken for virus infections if negative bacteriological cultures were accepted at face value

American consciousness of toxoplasmosis appears to stem primarily from the work of Sabin and Olitsky who rediscovered the organisms involved in 1935 during the course of their study of neurotropic viruses Their contact and association with Wolf and his colleagues led to the recognition of *Toxoplasma* in spontaneous human encephalitis These reports first appeared in 1937 and from that point on except for the interruption associated with the outbreak of World War II the number of references suggested that toxoplasmosis was becoming an American epidemic disease This statistical epidemic reached Europe and the rest of the world following the cessation of hostilities when investigators abroad had an opportunity to return to productive work

Today toxoplasmosis is appreciated in more and more public health and veterinary connections The importance of the disease in these two great and interrelated fields is ample justification for the publication of these paper

TABLE 1

HARVEST OF TOXOPLASMA FROM ROLLER TUBE TISSUE CULTURES

| Hours | Number mice dead of 5 inoculated with supernatant diluted to | | | | | Count of parasites per ml | Cytopathic effect |
|---|---|---|---|---|---|---|---|
| | $10^{-1}$ | 10 | $10^{-}$ | $10^{-}$ | $10^{-5}$ | | |
| 36 | 5 | 0 | 0 | 0 | 0 | 23 × 10 | 0 |
| 40 | 4 | 0 | 2 | 0 | 1 | 48 × $10^{4}$ | 0 |
| 44 | 5 | 5 | 2 | 0 | 0 | 13 × 10 | 1+ to 2+ |
| 48 | 5 | 5 | 0 | 0 | 0 | 28 × $10^{5}$ | 2+ to 3+ |
| 52 | 5 | 5 | 2 | 1 | 1 | 41 × 10 | 3+ |
| 56 | 5 | 5 | 2 | 1 | 0 | 28 × 10 | 3+ |
| 60 | 5 | 5 | 4 | 4 | 0 | 19 × $10^{5}$ | 3+ |

Tiss lt e w 21 lay-old fibrobl t iated th m nced mouse mbry nd maint ed th same med um described ... Ea h m se w noc lated w th 0 25 ml f th d l ted supernat mo ed from th ul u es t th t m des gnated Th hours rep ese t th pe iod ft oc lation f th lt es w th 1 m llion RH T xopla ma f m m se pe t eal dat

TABLE 2

THE SURVIVAL OF THE RH STRAIN OF TOXOPLASMA IN TISSUE CULTURE MEDIUM IN ROLLER TUBES

| Hours | Number of mice dead | Survival time |
|---|---|---|
| 0 | 5 | 8 8 9 9 18 |
| 1 | 5 | 8 9 9 9 10 |
| 5 | 5 | 9 9 9 9 10 |
| 12 | 1 | 18 |
| 24 | 0 | — |

F mic inoc lat d in ea h gro p Ea h m se oc ed 0 5 ml f T xopla ma pe so which w p epared t tain 60 parasites pe ml f med um Th med m ... Ea l sal ( 5 ml) Tra m (25 ml) nd 50 pe t f hick embry ... (2 ml) pl 50 ... f pe ic ll nd 5 g f t ept my per ml

portion of nonviable forms The parasites that escape from infected cells do not have the capacity for long survival extracellularly in the tissue culture nutrients This is seen in TABLE 2 While the suspension of parasites in Earle s replacement medium remained infective for mice for 5 hours after 12 hours the parasites were hardly viable This experiment was done in roller tubes under the same conditions maintained for tissue cultures Since we have not observed any synchronicity in the rupture of infected cells and release of parasites there is likely always to be some death of parasites in the nutrient medium and even when the maximum cytopathic effect is attained the yield is relatively low in the proportion of viable forms At earlier stages in the tissue culture prior to maximum cytopathogenesis the explants themselves should contain large numbers of viable parasites Some method of discarding the supernate and of releasing intracellular parasites in the explants might provide good yields of toxoplasmas all viable This method will have to be devised before tissue cultures can be used for such a purpose as for instance providing parasite suspensions for the *in vitro* dye test Perhaps stationary cell cultures would be better in these respects

Despite this disadvantage however tissue cultures of *Toxoplasma* are useful for a number of purposes Vischer and Suter (1954) used their macrophage

# PROPAGATION, MORPHOLOGY AND BIOLOGY OF TOXOPLASMA

By Leon Jacobs

*Laboratory of Tropical Diseases, National Microbiological Institute, National Institutes of Health, United States Department of Health, Education and Welfare, Public Health Service, Bethesda, Md.*

The broad scope afforded by the title of this paper allows a survey of many of the characteristics of *Toxoplasma* as they relate to the various aspects of toxoplasmosis which will be discussed by others. We shall consider the subjects in the order in which they are listed in the title.

### *Propagation*

*Toxoplasma*, despite its numerous puzzling features, does afford us the definite advantage that it is easily handled in the laboratory. So long as we remain aware of the fact that the ease of these artificial means of maintenance may jeopardize our recognition of some characteristics of the parasite as it occurs in nature, we can profit substantially from the facility with which *Toxoplasma* may be propagated.

*Tissue culture.* The fact that it is an intracellular parasite has little disadvantage because of presently available tissue culture methods. While it will not grow in medium without living cells, it is cultivable in a wide variety of cell types. It was first cultivated in Maximow slides of chick embryonic tissue by Guimaraes and Meyer in Brazil in 1942. We next used in our laboratory Maximow cultures of chick embryonic heart muscle, leg muscle, or liver epithelium (Jacobs, 1952). Since then other authors have used roller tube cultures of a number of different cells. For example, Chernin and Weller (1954) used mouse embryonic tissues and human epithelium, myometrium, and embryonic skin and muscle. Lock (1953) used embryonic rat heart muscle. In the past few years we have used minced mouse embryonic tissues and standardized cell cultures of monkey kidney epithelium. Vischer and Suter (1954) used cultures of macrophages from various laboratory rodents. *Toxoplasma* has also been grown in HeLa cells, and it is most likely that it will be found easily cultivable in many other types of cell as well.

The wide variety of cells in which the parasite can be cultivated *in vitro* is indicative of the extent to which it can be distributed in the tissues of animals with acute toxoplasmosis.

*Toxoplasma* in tissue culture manifests certain characteristics that require that it be handled differently from viruses. The yield of parasites from tissue cultures is not as great as might be hoped, nor is it easy to decide on an optimal harvest time. We have attempted to ascertain optimal times of harvest of cultures and the proportion of viable parasites in each yield. The results on one such test on roller tube cultures are presented in TABLE 1. It can be seen, first, that the number of toxoplasmas present at any time in the supernate of a tissue culture is not great compared to the numbers that can be recovered from the exudates of animals. Second, a comparison of the direct counts of the parasites with the infective titer of the supernates indicates that there is a high pro

easily from the liver, spleen and lung. In the later stages of the infection parasites are only rarely detectable in the extraneural viscera but are found easily in the brain. Ruchman and Fowler (1951) reported consistent recovery of *Toxoplasma* from the brain of rats infected for as long as two years. We have similar records in our laboratory for up to 24 months. Thus the rat serves as a bank for storing *Toxoplasma* until needed. This should be a good tool for teachers who require the parasite only occasionally for demonstration purposes.

*Preservation.* As a matter of interest, other banks that might be useful for storing *Toxoplasma* are tissue cultures at room temperature and hibernating animals. Meyer and de Oliveira (1943, 1945) kept Maximow slide cultures of *Toxoplasma*-infected chick embryonic tissue at room temperature. The parasites after 1 year and 8 months of such maintenance were as infective and virulent as the original strain from which they were derived. Rodhain (1951) infected a hibernating marmot intraperitoneally with a squirrel strain of *Toxoplasma*. The animal succumbed to the infection 18 days after the end of its sleep, which had lasted 3 months.

Eyles, Coleman and Cavanaugh (1956) and Weinman (1956) have recently developed the technique of preserving parasites by freezing in glycerine solutions. There is certainly attrition of the toxoplasmas in such preparations, but nevertheless enough organisms remain viable so that they can readily be recovered by inoculations of the thawed material into mice. This is certainly the most convenient method of preserving stains. Since, as will be described later, the maintenance of parasites by continuous passage in laboratory animals sometimes results in changes in their behavior, these banking procedures may be of more than academic interest in preserving the original characteristics of strains.

The chick embryo is also a useful tool for the primary isolation of *Toxoplasma* and for the maintenance of strains. Primary isolations have been made in chick embryos from ocular tissues and lymph node material. Occasionally, however, strains of the parasites are found that have been difficult to establish in chick embryos. The parasite, once established, can be maintained readily by weekly passages using the technique of yolk sac inoculation. When young embryos (7-day) are used, the development of calcified plaques on the chorioallantoic membranes is only occasionally seen; this is more likely to be found with 12-day or older embryos. As will be demonstrated later, parasites appear to alter less in virulence in this host than in the mouse.

## *Morphology*

*Toxoplasma gondii* in all of these situations presents the same morphology (FIGURE 1). It is a crescentic or arc-shaped organism with one end attenuated and the other more rounded. It measures about 2 to 4 μ in width and 4 to 7 μ in length. There is no centrosome or kinetoplast visible, but the cytoplasm may contain some chromatic granules. Some granules give a red staining reaction with the periodic acid Schiff reagent and are considered glycogen. The nucleus, as seen in Giemsa-stained preparations, appears as originally described by Nicolle and Manceaux (1908), a mass of granules of chromatin in a rather loose network. Cross (1947) has described a vesicular nucleus with chromatin

cultures to describe the role of cells and humoral factors in immunity to the infection. We have put tissue cultures to use in determining the minimal effective concentrations of pyrimethamine and sulfadiazine that cause inhibition of growth or complete destruction of proliferating toxoplasmas (Cook and Jacobs, 1956). It is possible, also, that tissue cultures will be useful in furnishing antigens for skin testing that will not have the disadvantage of antigens prepared from animals in that they can be made from human cells and in the absence of any contaminating agents. The use of such cultures for physiological studies on the metabolism of the parasites, or on the altered metabolism of host cells, is also an obvious possibility.

Beyond these uses, tissue cultures definitely afford an advantageous method for the primary isolation of parasites from suspected cases of toxoplasmosis. We have been successful in isolating toxoplasmas in tissue cultures inoculated with ocular tissues from a case of chorioretinitis (Jacobs, Fair, and Bickerton, 1954) and, more recently, with lymph node material from a case of toxoplasmic lymphadenopathy. Such isolations remove all doubt of the source of the toxoplasms in contrast to inoculation of animals, which must be proved to be free of spontaneous infection. They may, possibly, allow the maintenance of the parasite at the same level of virulence as it had in its original host; this point still requires investigation. Tissue cultures also allow isolation without need for adaptation of the parasite to a laboratory host.

*Maintenance in laboratory animals.* The ease of propagation of *Toxoplasma* in laboratory animals requires mention only for the uninitiated. Laboratory strains of *Toxoplasma* are easily passed by any route of inoculation in mice, rats, hamsters, rabbits, guinea pigs, chick embryos, and pigeons. The mouse is by far the most convenient animal, and has the advantage also that it is very unlikely to have spontaneous infections. Attempts by Sabin (1941), by Perrin (1943), and by our laboratory have failed to reveal any instance of natural infection with *Toxoplasma* in the laboratory mouse. The one recorded instance (Mooser, 1950) of spontaneous infection in this animal has been explained by Sabin (personal communication) as possibly due to the fact that the mice were received from a breeder who had attempted to rejuvenate his stock by the introduction into it of some wild mice to improve the vigor of the colony. In most instances *Toxoplasma* from human sources have proliferated readily in laboratory mice. In a few instances, however, strains of the parasite from human and other sources have required some adaptation to this animal.

The larger laboratory rodents, such as the hamster, guinea pig, and rabbit, have occasionally been found to have spontaneous *Toxoplasma* infections. If young animals are consistently negative serologically, however, there should be little reason to suspect natural infection in them. Since these animals show high susceptibility to *Toxoplasma* strains virulent for mice, they are not particularly valuable for maintenance purposes. The laboratory rat is a better animal in this respect. Here again, while spontaneous infections have been found in rats, young laboratory reared animals that are serologically negative are useful. The rat suffers little disease even when inoculated with very large numbers of parasites. In the early stages of the infection toxoplasmas are found in small numbers in the blood (J[illegible] F[illegible] and Jones) and can be isolated

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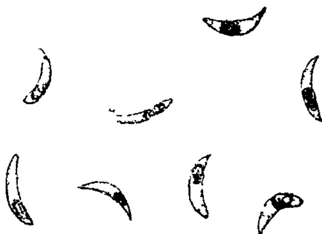
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F GURE 1 D awing of T plasma g d in G msa st in d sm f mo e pe t eal e date

particles arranged peripherally on its membrane The electron microscope studies of Gustafson *et al* (1954) support her observation that there is a definite nuclear membrane with a dense irregular layer of material applied to the inner surface They found masses of material of similar density however within the nucleus which varied considerably in their size and position Some clumping of dense material was seen that might indicate a consistent central mass possibly a nucleolus Holz (1954) using parasites obtained from colchicine treated mice describes a metaphase with six chromosomes

Variations in the shape of the organism may be related to the stage of reproduction or to the method of fixation Oval or rounded forms may be considered preliminary to cell division Air dried films usually show the parasite as more robust than wet fixed preparations in which the elongate appearance of the parasites is more like that seen in fresh preparations (FIGURE 2)

The use of the electron microscope has added considerable information regarding the morphology of *Toxoplasma* Various authors have described in preparations examined by ordinary light such structures as flagella (Splendore 1913) or a cytostyle (Cross 1947) These observations have not been confirmed by others The electron microscope allows a more definitive answer to the question of the presence of locomotor organelles ( u t tson *et al* using sections of fixed mouse peritoneal exudates cut to 0 05 μ r less have made a beautiful contribution in th s respect They describe at the anterior end of the parasite a short truncate hollow cone which they have de ignated a conoid The base of the conoid is open to the adjacent cytoplasm and the distal end is closely associated with the cell membrane (FIGURE 3) Associated with the proximal end of the conoid there appears a variable number 14 to 18 f homogeneous fibrils uniformly circular in cross section whi h diverge from the base of the conoid and extend toward the blunter end of the ell These they call toxonemes and consider them unique for *Toxoplasma*

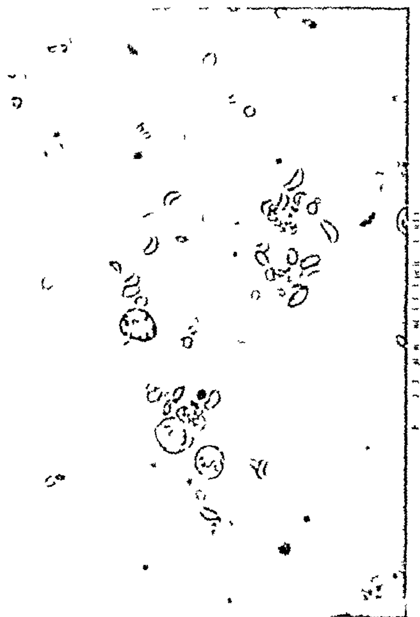


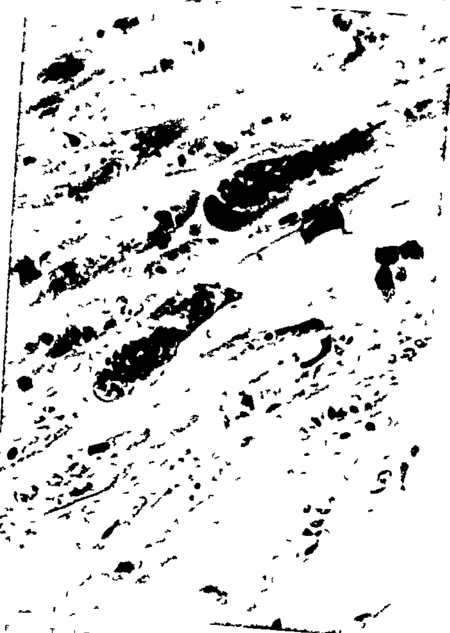


FIGURE 1 Drawings of *Toxoplasma gondii* in Giemsa stained smear from mouse peritoneal exudate

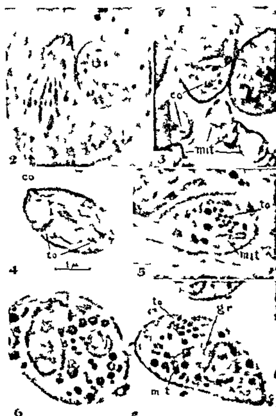
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F T l m ll k embry l ep h l m N t and idual pa rs of para




FIGURES [illegible] Electron micrographs of sections of *Toxoplasma gondii* (Gustafson *et al.* 1954 Am. J. Trop. Med. Hyg. 3: 1008–1021)

They consider it possible that the conoid may serve as a mouth structure or penetration device or be a vestigial cytopharynx. It is possible also that the organelle is subject to some movement. It is described as extended in some specimens and retracted in others. One can also conjecture that the toxonemes serve the function of retracting the organelle. It is not clear whether or not the distal end of the conoid is perforated. If it were, various explanations could be given for the occasional report of a flagellum or rod shaped structure at the attenuated end.

These authors have also described a longitudinal arrangement of fibrils in or on the cell membrane of *Toxoplasma*. Similarly, Bringmann and Holz (1954) studying intact *Toxoplasma* with the electron microscope describe the presence of a fibrillar network arising at the attenuated end of the parasite and extending on its surface for two thirds of the body length. These fibrils are interpreted as locomotor organelles that give rise to the gliding movement of the parasite.

In our studies of tissue cultures of the parasite, the toxoplasmas are frequently seen in vacuoles (FIGURE 4). The parasites are found paired, indicating that the method of reproduction is by longitudinal division. Eventually

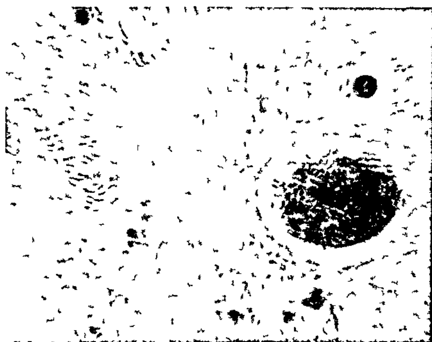


FIGURE 6 Pseudocyst of *Toxoplasma gondii* [illegible] chronically infected [illegible]

the proliferating forms may completely fill the host cell (FIGURE 5) rupture the cell wall and disperse to invade others There is reason to differentiate these proliferative forms from the cystlike agglomerations of parasites that are seen in the chronic stage of infection (FIGURE 6) The individual parasites in the proliferative stages according to Frenkel and Friedlander (1951) have few small glycogen granules while those in the cyst form have many larger granules The size of the host cell is enlarged in the cyst form and the wall of the cyst undergoes some changes It becomes argyrophilic and weakly PAS positive According to Rodhain (1950) and Rodhain and Cerebetzoff (1951) it is resistant to pressure and is elastic Cross (1947) stated that parasites within cysts required a longer time for hydrolysis in staining by the Feulgen technique than did free parasites It is not clear however that she was actually dealing with the pseudocyst form but rather with the intracellular proliferative forms

The question arises as to the origin of the cyst wall On the assumption that the wall is the remains of the host cell wall with no contribution from the parasite the cystlike structure has been designated a pseudocyst Frenkel and Friedlander believe that because of its staining reactions it is likely that the wall may be derived from the parasite and that the word cyst is appropriate It is here that the observations on the localization of toxoplasmas in vacuoles of infected cells are pertinent Gustafson *et al* report that in the space

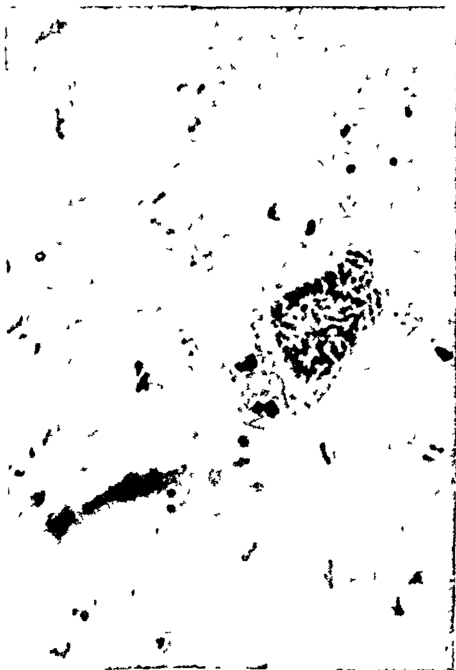


FIGURE 5. M ss f t [illegible] la ma h k [illegible]

at least a month Vermeil and Maurin (1953) recovered a *Toxoplasma* strain of human origin from chameleons (*Chamaeleo vulgaris*) and geckos (*Tarentola mauritanica*) for up to 15 days after inoculation We can now add to this list the painted turtle (*Chrysemys picta*) and we have confirmed Frankel s findings on *Terrapene carolina* (Jacobs and Melton to be published) Therefore it appears that some of the morphological reports of *Toxoplasma* in reptiles in nature may eventually be confirmed by isolation procedures

On the other hand attempts at infection of amphibia (Kozar 1952 Manwell *et al* 1953) have resulted thus far in failure

While originally many specific names were given to *Toxoplasma* from the various hosts biological and immunological studies have indicated that these parasites are members of the same species *Toxoplasma gondii*

*Course of infection* The lack of specificity of *Toxoplasma* for particular cells or cell types makes it possible to establish infection in animals by any route Following some proliferation at the site of entrance a parasitemia develops and the infection becomes generalized The parasitemia persists until and sometimes for a while after antibodies make their appearance in the blood stream This has been demonstrated in a number of laboratory hosts among them the rat rabbit chicken dog and pigeon (Jacobs and Jones 1950 Jacobs Melton and Cook 1953 1955) There is no consistent parasitemia after antibodies reach a significant level although occasionally the blood may be found positive after the acute infection has subsided Such findings are considered due to the occasional presence of a parasitized leukocyte in the blood The parasites within the host cell are protected from antibody Whether or not such parasites in a wandering cell could on release reestablish infection in a new site is a matter of considerable importance It is believed that these parasitemias probably occur only rarely during the latter part of acute episodes and that proliferation of parasites in tissues in the presence of significant amounts of antibody resulting from a previous infection is not very likely

Actually what is generally found in the period after the widespread dissemination of parasites and the production of lesions in many organs is first the clearing of the blood and then more slowly the disappearance of parasites from many of their tissue sites We must distinguish between parasitization of tissues and parasitemia in the tissues The former lasts longer than the latter and toxoplasmas can be found in the parenchyma cells of the liver for instance as well as in Kupffer cells and fixed macrophages after they are no longer demonstrable in the blood Data on this are available from a variety of hosts such as rats dogs and chickens For example Ruchman and Fowler (1951) reported that in rats infected with RH toxoplasmas parasites were found in the spleen for 2 weeks in the liver and lungs for 10 weeks and in the brain for 2 years after infection Parasites were demonstrable in the blood regularly during the first week after inoculation and then occasionally during the next 9 days The data in TABLE 3 summarize similar studies on dogs (Jacobs Melton and Cook 1955) and TABLE 4 shows additional information on chickens Even relatively early there is a gradual clearing of the tissues or at least a diminution in the numbers of parasites residing in them as evidenced by the

between the parasites and the vacuolar wall there is a fine filamentous or granular precipitate visualized by the electron microscope Also there are concentrations of host cell mitochondria at the edges of the vacuoles It is conceivable that, as the parasites increase in number and vacuoles enlarge the precipitates become applied to the host cell wall and contribute to its staining properties In contrast to the cysts of parasites such as *Sarcocystis* and *Besnoitia* which form more definite walls the origin of the cyst or pseudocyst wall of *Toxoplasma* is not clearly parasitic

*Biology*

It is obvious from the foregoing discussion that *Toxoplasma* is a remarkably indiscriminate organism parasitizing many different types of cells and many hosts The organism is widely distributed in nature and there are numerous reports of its occurrence in many orders of mammals and birds

*Distribution in nature* Its host range among the mammals is from the most primitive forms to the most highly developed There is a morphological report (Coutelen 1932a) of its occurrence in a marsupial the wombat Among Insectivora it has been found in the mole (von Prowazek 1910) and has also been shown to infect the shrew and the hedgehog (Laveran and Marullaz 1913) Many species of rodents have been found infected in nature the gondi mouse rat rabbit guinea pig squirrel and marmot Among carnivores the dog fox cat and chinchilla have been found naturally infected Artiodactyla such as swine sheep and cattle are also hosts of the parasites (see Habegger 1953 or Callahan *et al* 1946 for citations of most of these reports also Keagy 1949 on the chinchilla Fankhauser 1951 on canine toxoplasmosis Moller 1952 on *Toxoplasma* in the fox Farrell *et al* 1952 and Sanger *et al* for 1953 for swine and cattle) *Toxoplasma* has been found in the baboon (Levaditi and Schoen 1933) the chimpanzee (Kopciowska and Nicolau 1938) the whiteface monkey *Cebus capucinus* (de Rodaniche 1954a) as well as in man In addition the marmoset *Marikina geoffroyi* and the night monkey *Aotus zonalis* have been experimentally infected (de Rodaniche 1954b) and rhesus monkeys are also susceptible (Sabin and Ruchman 1942 Cowen and Wolf 1945)

Among birds there is no doubt of the occurrence of *Toxoplasma* in pigeons (Jacobs Melton and Jones 1952) and in chickens (Hepding 1939 Ericksen and Harboe 1953) Other birds have been experimentally infected notably the canary and the duck (Wolfson 1941) the English sparrow the song sparrow and the canary (Manwell *et al* 1945) and the purple grackle (Manwell and Drobeck 1951) A large number of other birds have been reported to be infected (see Habegger 1953 for a listing of these) It is best however to reserve judgment on these records until biological evidence is obtained to confirm the microscopic findings that are their main basis

There are morphological records of *Toxoplasma* in reptile (Plimmer 1916 and Coutelen 1932b) Since no isolations of the parasite from reptiles have yet been accomplished these reports still stand unconfirmed Frenkel (1953a) however stated that lizards (*Sceloporus* and *Anolis*) and turtles (*Terrapene*) were susceptible to the RH strain of *Toxoplasma* and harbored the parasite for

persistent infection with *Toxoplasma* merit discussion in regard to the occurrence of congenital toxoplasmosis in humans There is little doubt that in most cases neonatal toxoplasmosis is the result of congenital transmission of the infection On the basis of clinical and serological findings it has been assumed that congenital transmission occurs only when the mother acquires the infection during the gestation period

On the other hand Weinman (1952) expressed the view that congenital human cases represent the transmission from a chronic tolerant carrier to the infant He presumes that pregnancy may result in an exacerbation of latent toxoplasmosis and the spread of parasites from a localized site of chronic infection to the placenta and fetus.

Reports from Germany by Hellbrugge (1953) and Wildfuhr (1954) indicate that rats with chronic infections have transmitted toxoplasmosis to their offspring Hellbrugge found no evidence of change in the placental tissues indicating proliferation there Furthermore the recent work of Sanger *et al* (1955) reports that sows with asymptomatic infections revealed only by the presence of low dye test titers have given birth to infected pigs Campbell (1953) in England claims that there has been continued congenital transmission of *Toxoplasma* from one mother to her children albeit the evidence is poor A report from Austria (Seitelberger and Spiel 1953) makes a similar claim There are also case reports of ocular disease presumed toxoplasmic with exacerbations during pregnancy (Johnson 1946) although in these cases the infants were born without toxoplasmosis

There are some bases for discounting this argument Cowen and Wolf (1950) stated that toxoplasmas did not appear to pass the placental barrier except after local proliferation in the maternal uterine tissues Eichenwald (1948) did not find congenital transmission in chronically infected mice Second studies on the challenge of immune animals has shown little likelihood of proliferation of parasites in the presence of antibody Third no technique such as splenectomy or irradiation has been found to result in exacerbation of chronic infection (Weinman 1943 Frenkel *et al* 1952) Furthermore cortisone given to chronically infected mice in doses sufficient to cause death of some of the controls did not result in reactivation of latent infections (Jacobs and Melton 1953) Cortisone did not increase the parasitemia in rabbits infected with our strain of low virulence (113 CE) nor did it result in a significantly higher occurrence of chronic encephalitis in mice given doses of the drug in the period between the cessation of the disseminated stage of infection and the usual time of onset of encephalitis

There is also the report by Gard and Magnuson (1951) of the occurrence of lymphadenopathic toxoplasmosis in a young woman with symptoms appearing about two weeks after the start of pregnancy Diagnosis was made by rising antibody titers which were slow in appearing The baby was born at term without any evidence of disease and with passively transferred antibodies that decreased in titer with time Here apparently either because of low invasiveness of the parasite strain or because immunity developed in the mother before the fetus was infected congenital transmission did not occur

It appears justifiable to assert that except under unusual circumstances there

TABLE 3

DEMONSTRATION OF PARASITES IN DOGS SURVIVING PARENTERAL RH INFECTION *

| Age at time of infection | No of dogs | Time of sacrifice | No of dogs | | Organs positive | Survival days of inoculated mice |
|---|---|---|---|---|---|---|
| | | | Positive | Negative | | |
| 45 days or less | 4 | 1 mo–2 5 mos | 0 | 0 | | |
| | | 9 mos–10 mos | 2 | 0 | Brain | 8–9 |
| 65 days | 1 | Over 2 years | 1 | | Liver | 9 |
| 4 mo | 5 | 1 week | 1 | 0 | Lung | 6–7 |
| | | | | | Liver | [illegible]–8 |
| | | | | | Spleen | 5–7 |
| | | 2 weeks | 1 | 0 | Lung | 9 |
| | | | | | Liver | 9 |
| | | | | | Spleen | 9 |
| | | 9 mos or more | 0 | 3 | | |
| 7 to 9 mos | 2 | 3 mos–10 mos | 0 | 2 | | |

* All [illegible] 4 dogs were infected with 10 000 RH parasites [illegible] only The others were infected as follows 1 of those infected at 45 days of age received 5 000 RH parasites intradermally The dog infected at 65 days of age received 2 million parasites [illegible] only The dogs infected at 7 and 9 months of age received 100 000 parasites intradermally [illegible] respectively

longer survival time of mice inoculated with organ suspensions at progressively later periods

*Residual infection* Despite this clearing of the tissues however the presence of residual infection has been demonstrated in animals long after experimental infection For example, we have found a dog infected about 2 years previously still to have toxoplasmas in the liver (TABLE 3) By far the easiest site from which to isolate parasites in latent infections is the brain As already mentioned rat brains are almost consistently found positive for as long as 2 years after experimental infection We have similarly found the brain of a dog positive 10 months after inoculation and the brains of pigeons were found to harbor toxoplasmas 3 years after experimental inoculation and after antibodies were no longer demonstrable in the peripheral blood On occasion the heart of experimental animals has been found to harbor parasites after these organisms have been cleared from other organs

Descriptions of chronic disease in animals indicate that these persistent parasites have a continuing pathogenicity Levaditi *et al* (1929a) described chronic encephalitis in experimentally infected rabbits and mice Weinman (1943) and Weinman and Berne (1944) also reported encephalitis in mice that survived infection with small numbers of parasites or were protected from death by treatment with sulfonamides Frenkel (1951) reported progressive encephalitis in the golden hamster and we have described late death of mice from encephalitis after the inoculation with a strain of low virulence (Jacobs and Melton 1954)

The presence of *Toxoplasma* like bodies in heart skeletal muscle and the eye of humans was described by Kean and Grocott 1947 Syverton and Slavin 1946 Plaut 1946 and Wilder 1952 and has been substantiated by isolation of parasites from the heart and eye by Cathie (1955) and by Jacobs Fair and Bickerton (1954)

*Circumstances of congenital infection* The implications of these findings of

Strains that have been isolated from nature show wide variation in their virulence Parasites isolated from severe cases of human toxoplasmosis are generally highly virulent for mice This is not always so however For example Armstrong and MacMurray (1953) reported a lapse of 54 days before appearance of symptoms in mice inoculated with lymph node material from a severe case of acquired toxoplasmosis

Strains isolated from latent infections in animals are frequently low in virulence for mice For example a strain isolated from a dog by T L Perrin and transferred to us produced only chronic infections in mice and was not recovered from mice until two months later Kodhain (1950) has described strains isolated from squirrels that produced only chronic infections in mice two of these strains were lost in mouse passage We have previously recorded (Jacobs Melton and Jones 1952) the isolation of strains from pigeons requiring numerous blind passages in mice before they were easily demonstrable De Rodaniche has stated that in early passages in mice of a *Toxoplasma* strain isolated from the whiteface monkey *Cebus capucinus* a chronic type of infection was produced A number of other examples can be cited

Whether or not this difficulty of establishing strains in mice represents an adaptation of the parasite to the mouse or a general increase in virulence requires some comment On the whole strains virulent for mice such as the RH strain show the greatest virulence for other laboratory animals such as the guinea pig hamster and rabbit and for pigeons and chickens Another criterion of virulence can be used here the parasitemia in nonfatal infections For example pigeons infected with the RH strain showed a much higher and more consistent parasitemia than those infected with a chinchilla strain or with our strain 113 CE even when they survived infection (Jacobs Melton and Cook 1953)

Strains maintained in laboratory mice generally increase in virulence not only for mice but for other hosts The enhancement in pathogenicity of an avirulent strain maintained in mice as compared with the same strain maintained in the chick embryo has already been described (Jacobs and Melton 1954) *The same enhancement of virulence has occurred on an additional oc*casion when we have taken the chick embryo-passaged line and transferred it serially through mice (Melton and Jacobs 1955) Others have similarly observed such increases in virulence De Rodaniche s monkey strain already mentioned at first produced chronic infections but after some 24 passages in mice was reported to be highly virulent for them De Rodaniche (1954b) has also reported that the marmoset (*Marikina geoffroyi*) was highly susceptible to the strain after only two passages in mice and that after the first passage in the marmoset it was greatly increased in virulence for mice

Thus enhancement in virulence does not occur in relation to a single host species but is general It may be associated with the artificial means of propagation of the parasite used in the laboratory or to the selection of more rapidly multiplying forms in continuous passage Sabin and Olitsky (1937) described a sudden change in the ability of a guinea pig strain to destroy host cells Such an occurrence would unconsciously be followed by maintenance of more rapidly destructive parasites in successive passages It is interesting that this change

is little likelihood of a mother giving birth to more than one toxoplasmic child in separate pregnancies This is supported by the histories gathered by Eichenwald and by Feldman (elsewhere in this monograph) on the mothers of toxoplasmic infants One cannot definitively rule out, however, the possibility that under peculiar conditions a woman might have successive pregnancies complicated by toxoplasmosis It is probable that we are dealing here with the multifaceted problem of host susceptibility and variation in virulence of parasite strains

*Strain variation* We have already reported in abstract form some differences among inbred strains of mice in response to infection with our strain 113 CE (Jacobs and Melton 1953) The major point to be noted is that the differences in the mouse strains were not discernible when tested against highly virulent parasites (our work with the RH strain and that of Christen and Thiermann (1954) with RH) It was only when the parasites were relatively low in virulence that the mouse strains were found to react differently

The main characteristic on which parasite strains have been differentiated is their virulence for mice and sometimes for other laboratory animals The criteria available for such evaluations are the mortality of animals inoculated by various routes with graded doses of the parasite the symptoms produced and the survival time of the mice so inoculated Harboe and Erichsen (1955) in Norway have recently described also a length difference between two strains of considerably divergent virulence with the larger parasites (RH) being associated with the greater virulence They found a number of intermediate strains not distinguishable in this respect

TABLE 4
DEMONSTRATION OF TOXOPLASMA IN THE TISSUES OF CHICKENS EXAMINED AT WEEKLY INTERVALS AFTER INFECTION WITH THE RH STRAIN

| Chick No | P ra t mia | | D y f sa ifc | T ssu pos ti e | S r l days f inoculated mic † |
|---|---|---|---|---|---|
| | Day tested | r l day f oc lated m † | | | |
| 88 | 4<br>/ | —<br>11 | / | Liver<br>Spleen<br>Lung<br>Heart<br>Kidney<br>Bone marrow | / /<br>4 7<br>3 8<br>7 7<br>9 9<br>9 9 |
| 05 | 8<br>11 | —<br>— | 14 | Spleen<br>Heart | 9 9<br>8 8 |
| 9 | 4<br>8 11 15 22 | 8 12<br>— | 21 | Heart<br>Breast muscl | 10 10<br>10 10 |
| 19 | 4<br>11<br>8 15 22 | 10 10<br>10 10<br>— | 25 | None | — |

Ch k w s week old h n nfected Th y f d t ly th 5 000 RH *T pla*
T ss es tested w h pl l ng heart k d be m r b m l and l g m scl
† T o m e o lated trape t lly ea h t Th r gu g th s rs l t mes of the
i d idu l mic A d h d tes g t e lt

Strains that have been isolated from nature show wide variation in their virulence Parasites isolated from severe cases of human toxoplasmosis are generally highly virulent for mice This is not always so however For example Armstrong and MacMurray (1953) reported a lapse of 84 days before appearance of symptoms in mice inoculated with lymph node material from a severe case of acquired toxoplasmosis

Strains isolated from latent infections in animals are frequently low in virulence for mice For example a strain isolated from a dog by T L Ferris and transferred to us produced only chronic infections in mice and was not recovered from mice until two months later Rodhain (1950) has described strains isolated from squirrels that produced only chronic infections in mice two of these strains were lost in mouse passage We have previously recorded (Jacobs Melton and Jones 1952) the isolation of strains from pigeons requiring numerous blind passages in mice before they were easily demonstrable De Rodaniche has stated that in early passages in mice of a *Toxoplasma* strain isolated from the whiteface monkey *Cebus capucinus* a chronic type of infection was produced A number of other examples can be cited

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Strains maintained in laboratory mice generally increase in virulence not only for mice but for other hosts The enhancement in pathogenicity of an avirulent strain maintained in mice as compared with the same strain maintained in the chick embryo has already been described (Jacobs and Melton 1954) The same enhancement of virulence has occurred on an additional occasion when we have taken the chick embryo-passaged line and transferred it serially through mice (Melton and Jacobs 1955) Others have similarly observed such increases in virulence De Rodaniche's monkey strain already mentioned at first produced chronic infections but after some 24 passages in mice was reported to be highly virulent for them De Rodaniche (1954b) has also reported that the marmoset (*Marikina geoffroyi*) was highly susceptible to the strain after only two passages in mice and that after the first passage in the marmoset it was greatly increased in virulence for mice

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has not occurred in chick embryos, and this is suggestive of the possibility that it would be profitable to investigate the stability of strains in tissue cultures The preservation of the original characteristics of *Toxoplasma* as found in nature may be necessary for successful studies on the life cycle of the parasite

*Immunity* Generally a high degree of immunity develops in animals that survive toxoplasmosis This is true except in mice, in which considerable differences in the degree of immunity have been reported While others have reported that mice surviving one inoculation with *Toxoplasma* were immune to subsequent challenges (e g Weinman 1943 Frenkel, 1952 Biocca 1945 Rodhain 1950 Ruchman and Johansman 1948 Wolf Cowen and Paige, 1939) with the strains in use in our laboratory mice rarely develop a lasting immunity The main evidence of an immune response in mice is an increase in survival time The longer the delay between initial infection and challenge the less is the extension of survival time (Jacobs and Melton 1955) When mice immunized with 113 CE do survive challenge with the RH strain as occasionally occurs, the RH strain can be demonstrated as established in the tissues This is in line with Rodhain s (1950) observations in immune mice that survive challenge

The multiplication of parasites introduced by a challenge inoculation into larger rodents or birds that have survived an initial infection is of rarer occurrence A number of workers have reported fast immunity to challenge in such animals, with failure to recover parasites introduced in a challenge inoculum Such reports date back to Levaditi *et al* in 1928

Immunity is related to the production of neutralizing antibodies demonstrable in neutralization tests and requiring for their activity the presence of a heat labile serum component On the basis of a similarity in the requirements of the neutralization test (Sabin and Ruchman 1942) or the dye test of Sabin and Feldman (1948) it is believed that both demonstrate the presence of the same antibodies

Levaditi *et al* (1929b) concluded that immunity in toxoplasmosis was probably cellular because they could not demonstrate any antitoxoplasmic activity in the serum of an immune animal It is probable that this was due to loss of the heat labile component of the serum There is evidence however that both cellular and humoral factors are involved in the immune response

It has been possible to produce a degree of immunity in guinea pigs by the intramuscular or intraperitoneal injection of antigens consisting of toxoplasmas killed by low heat, formalin or phenol The immunity is manifested by the development of antibodies demonstrable by the dye test and by the resistance of the guinea pigs to challenge inocula that kill control animals in about 9 or 10 days (TABLE 5) The immunity however is of the type described above in mice There is a dissemination of the parasites which are demonstrable after challenge in various organs depending on the time of sacrifice but longer in the brain than elsewhere

Passive immunization of rabbits by intravenous injection of large quantities of immune rabbit serum has resulted in the production of relatively high levels of circulating antibody Such rabbits have died however following challenge inoculation as rapidly as controls and have shown similar parasitemias Each

TABLE 5

IMMUNITY INDUCED BY DEAD ANTIGENS

| No. of guinea pigs in group | [illegible] | Results of challenge | | | | |
|---|---|---|---|---|---|---|
| | | No. died | Distribution of parasites | | | |
| | | | Brain | Liver | Spleen | Fetus |
| 3 | 10% toxos in saline† (heated) | 0 | + (7)‡ | 0 | 0 | |
| | | | + (7 8) | + (16) | + (16) | |
| 3 | 10% toxos in 4% formalin saline | 0 | + | 0 | 0 | |
| | | | + | 0 | 0 | |
| 3 | 10% toxos in 0.5% phenol saline | 0 | + (8 9) | 0 | 0 | |
| | | | + (8 9) | + (9) | + (9) | + (4) |
| 2 | Saline | 2 (10) | | | | |
| 5 | 10% toxos in saline (heated) | 0 | + (8 10) | 0 | | |
| | | | + (7 8) | 0 | | |
| | | | + (7 ) | 0 | | |
| 4 | Saline | 4 (9 9 [illegible] 10) | | | | |

[illegible] and RH [illegible]

[illegible] challenge

† The [illegible] 10 per cent ([illegible]) [illegible] parasites [illegible] intramuscularly to the [illegible]

‡ Figures in parentheses indicate [illegible] days [illegible] T om [illegible] 0.5 ml [illegible] 1 10 [illegible] the [illegible]

enwald (1949) in studies on the effects of antiserum and sulfadiazine in experimentally infected mice stated that while the serum or the drug individually had some beneficial action the combination of these substances was considerably more effective than either used alone In so far as the author is aware no other studies on passive immunization have been performed

The studies of Vischer and Suter on cultivation of *Toxoplasma* within macrophages in vitro are interesting in this respect Macrophages from immunized animals cultivated with immune serum exhibited the greatest capacity to inhibit proliferation of *Toxoplasma* Neither immune cells with normal serum nor normal cells with immune serum showed similar inhibitory properties Coupled with the observations described earlier these findings indicate the likelihood that both humoral factors and educated cells are responsible for immunity to *Toxoplasma*

Whether due to differences in cell types within the nervous system compared to the extra neural viscera or to less diffusion of humoral antibodies into the central nervous system it is undoubtedly true that the brain and eye represent the most frequent sites of persistence of toxoplasmas in animals and probably in man Possibly because of low immunity in the ocular fluids and tissues rupture of pseudocysts and proliferation of parasites may occur from time to time resulting in exacerbations of chorioretinal lesions (Frenkel 1951b Frenkel and Friedlander 1951 Jacobs Cook and Wilder 1954 Jacobs Fair and Bickerton 1954 Woods Jacobs Wood and Cook 1954)

*Serology* The humoral antibodies demonstrable by serological test are of two types complement fixing and cytoplasm modifying (dye test) antibodies These antibodies can be distinguished by their time of appearance and persistence and by their lack of relationship in titer in some instances The dye

test antibodies, as has been mentioned above appear to be neutralizing anti bodies The mechanism of the dye test has, indeed been interpreted by Lelong and Desmonts (1952) as a partial lysis of the parasites, a modified Pfeiffer phe nomenon, whereby the affected organisms lose those constituents of the cyto plasm that are ordinarily stained by methylene blue The heat labile compo nent of the reaction mixture characterized originally by Sabin and Feldman (1948) as distinct from complement because it could not be replaced by C 1 and C′2 after heating of the serum has been reported by Roth (1953) to be comple ment including the other two factors C 3 and C′4 * While these latter factors are relatively heat stable most of their activity can be lost after 30 minutes at 56° C (Kabat and Mayer, 1948) Therefore considering the relatively large amount of accessory factor serum required to effect the dye test reaction it is possible that after heating such serum the concentrations of C′3 and C 4 might be reduced below an effective level and that the reaction would not take place even with the replacement of the heat labile factors of complement We can add the observation that the chelating agent, Versene which binds calcium and magnesium can completely inhibit the dye test reaction in certain concentra tions

Since in recent years the dye test has been subjected to the criticism of lack of specificity (Muhlpfordt 1951 Awad 1954) we have run a number of tests in an attempt to obtain definite answers to this question

In experimental studies we have as yet found no definite evidence that *Toxo plasma* cross reacts serologically with *Trypanosoma cruzi* or *Plasmodium berg hei* in rats or with *Plasmodium gallinaceum* or *Eimeria tenella* in chickens Tests on a large number of squirrels found infected with *Hepatozoon* sp and on three rhesus monkeys with *Sarcocystis* have been negative for *Toxoplasma* antibodies Sabin (personal communication) has also found negative dye tests in some cyno molgus monkeys with *Sarcocystis* infections Moscovici (1953) also reported no cross reaction between *Toxoplasma* and sarcosporidia Rats showing low dye test titers of 1 1 or 1 4 are frequently found to harbor *Encephalitozoon* in the brain so there may be some cross reaction at very low titers between *Toxo plasma* and *Encephalitozoon* In some instances also rabbits infected with *Besnoitia jellisoni* show dye test titers up to 1 16 There is no cross-immunity between *Besnoitia* and *Toxoplasma* (Frenkel 1953b and our observations)

On the basis of this information it is believed that titers of 1 16 and over obtained in the dye test for toxoplasmosis are significant and that they prob ably indicate past exposure to *Toxoplasma*

*Data relative to transmission* Serological survey data in addition to reports of cases of toxoplasmosis or epidemics of the disease in various animals and in man indicate that *Toxoplasma* is one of the most ubiquitous and prevalent of parasites How then does toxoplasmosis spread from man to man among animals or from reservoir animals to man?

Toxoplasmas are found widely distributed throughout the body of acutely infected hosts They have been found in the blood urine feces milk and sa liva and in serous exudates from the conjunctiva of various animals in the late

It h l t m t th th tt t th t P l G f l d l d t fi d s rv f t
p p d pl C C 3 d C 4 (A E ptl M d B l F t k p bl h d)

stages of acute infections (see Jacobs 1953 for citations) The parasites thus found are proliferative forms with little resistance to the external environment

These parasites are rapidly destroyed by drying by changes in osmotic pressure by low heat and by freezing and thawing except in special glycerine solutions such as reported by Lyles Coleman and Cavanaugh (1956) and by Weinman (1956) There is rapid attrition of organisms standing outside of cells in saline or serum saline solutions (Jacobs Jones and Melton 1952) or in tissue culture nutrients, as was observed earlier There is also attrition of such proliferative forms in the carcasses of infected animals Such material stored in the refrigerator for two weeks may be still infective to mice but the survival time of the mice is definitely prolonged On the basis of the poor resistance of the proliferative forms to the external environment it is believed unlikely that the spread of the parasite takes place by any contaminative means There is only one record (Olafson and Monlux 1942) reporting the infection of one puppy by close contact with litter mates sick with toxoplasmosis In experiments performed in our laboratory this did not take place under similar circumstances (Jacobs Melton and Cook 1955)

There is evidence that the pseudocyst form of the parasite is more resistant to storage and to the action of digestive juices than the proliferative forms Thus while other workers (Adams *et al* 1949 van Thiel 1949 Cowen and Wolf 1950) reported only occasional success with attempts at producing infection by feeding mice the carcasses of acutely infected mice Lichenwald (1948) consistently produced infection by this means with tissues of chronically infected mice Similarly our results with feeding the tissues of mice dead or dying of toxoplasmosis show only little success Only 13 infections resulted from feeding 84 mice with such tissues despite the presence of large numbers of proliferating parasites On the other hand much more consistent results were obtained with the feeding of brains from rats with chronic infections (TABLE 6) On the basis of these observations it seems not unlikely that carnivores may acquire toxoplasmosis by consuming infected tissue The intestinal lesions seen in dogs cats and foxes with spontaneous toxoplasmosis may indicate that the gastrointestinal tract is the portal of entry

Whether or not we can extend this viewpoint to humans remains a matter of

TABLE 6
RESULTS OF FEEDING TISSUES FROM CHRONICALLY INFECTED RATS

| Rat N | N f m dead f 4 inoculat d | N m e d d N fed |
|---|---|---|
| 1 | 4(7 8 8 9)‡ | 2 4(8 9) |
| 2 | 4( 8 8 9) | 3 3(8 9 9) |
| 3 | 2(8 9) | 2 4(9 9) |
| 4 | 2(8 9) | 0 3 |
| 5 | 4(7 8 9) | 1 3(8) |
| 6 | 4( 7 9) | ? 4(8 8) |
| 7 | 4(8 8 9 9) | 1 4(8) |
| 8 | 4(8 8 8 9) | 2 4(9 9) |

R t fected k y by
I oc lated t 10 gt l )
† Th ee ( th g f d t ea h m
‡ ew in pa tes d t h l d y f th m

considerable doubt. Lesions of the intestinal tract have not been reported in acquired toxoplasmosis of humans. The findings of Cole and his co-workers of toxoplasmosis in swine and cattle, in addition to the earlier data on sheep have nevertheless stimulated investigations on the possibility that undercooked pork or beef may serve as a source of human toxoplasmosis. Our serological study of orthodox Jews (Jacobs, Cook, and Neumann, 1954) in the older age groups in which we found a prevalence of dye test reactions as high as that in the general population, rules out pork as the *sole* source of human infection. A similar study on Hindus would be valuable to evaluate the role of beef. The areas in which the highest prevalences of positive dye test reactions are found have populations, however, that only rarely consume meat (as in Tahiti) or do so only when it is well cooked (as in Guatemala).

Whether or not carnivores or omnivores can acquire toxoplasmosis in infected flesh, some explanation is still needed for the means of infection in herbivores and seed eaters. The transformation of the parasite in the gut into a resistant form that could serve as a means of spread to such hosts has been investigated by feeding the feces, fresh or stored, of infected animals or of animals fed infective tissue, to others, always with negative results. Since a parasitemia does exist in the acute stage of toxoplasmosis in all animals, the possibility that infection is spread by bloodsucking arthropods has been investigated by a number of workers. The results of such studies were summarized in an earlier review (Jacobs, 1953). In addition to the reports listed there, Havlík (1951) in Czechoslovakia states that toxoplasmas survived in the tick *Ornithodorus moubata* for 23 days. The tick bite did not transmit the infection, but fluid from the coxal glands and excrement of the ticks proved to be infectious 23 days after blood sucking. Another report by Giovannoni *et al.* in Brazil (1952) records that infection was acquired by *Culex quinquefasciatus* feeding on infected pigeons, and that the organism could be demonstrated in the mosquitoes for 96 hours. No transmission occurred by bite.

In general, most of the studies indicate like phenomena. Arthropods can acquire the infection by biting, and the parasites have been found to survive in arthropods for a number of days. In our own studies, the one possible case of transmission by arthropods occurred with *Dermacentor* (Woke, Jacobs, Jones and Melton, 1953), but *Rhipicephalus sanguineus* apparently harbored the parasite for at least 60 days, through two molts, as was demonstrated by our isolation of strain 113 from mice inoculated with the macerated ticks. It is our opinion, therefore, that continued study of this method of transmission with ticks and other arthropods should be prosecuted. The results of Havlík are especially encouraging. The observations of Giroud *et al.* (1953) and others on the development of toxoplasmosis in mice or other animals inoculated with ticks or mites collected in nature, although equivocal, add support to this thesis.

Epidemiological data on human toxoplasmosis have been provided by a number of surveys using the dye test and skin test (Frenkel, 1948; Feldman and Sabin, 1949; Feldman, 1953; Beverley, 1953; Bugi, 1951, 1953; Bustos Castro *et al.*, 1952; Gard, 1951; Gibson *et al.*, to be published; Harboe, 1952; MacDonald, 1950; Piekarski, 1952; Romana and Lifschitz, 1954; Roth, 1953b; Thalhammer, 1951). In addition to the general finding that there is an increasing

prevalence of positive reactions with increasing age certain salient facts are to be noted from these data (1) the prevalence of the infection is highest in areas such as Tahiti and the coastal region of Guatemala (2) the prevalence is low in areas such as Iceland and Alaska (3) the prevalence is higher in southern Sweden than in northern Sweden (4) more positive reactions are found among the population of the coastal regions of Mexico than at the high altitude around Mexico City (5) within the United States there are regional differences in prevalence with the eastern regions possibly showing higher rates than western areas (6) the rate among Navajo Indians in Arizona is surprisingly low while it is higher in their dogs These data may possibly be summarized by a statement that toxoplasmosis is more prevalent in warm moist areas than in cold or hot dry areas Such a generalization could be explainable on the basis of arthropod transmission but also perhaps on other bases as well At the present stage of our knowledge the pieces of the puzzle do not make a complete picture It serves no purpose at this time however to postulate multiple modes of transmission because this idea detracts from the enthusiasm with which the problem of the spread of *Toxoplasma* should be pursued without itself providing adequate or definite answers.

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# CONGENITAL HUMAN TOXOPLASMOSIS*

*By* Harry A Feldman and Louise T Miller

*Department of Public Health and Preventive Medicine State University of New York Upstate Medical Center at Syracuse N Y and the Kilian Research Laboratory Witting Johnson Hospital for Rheumatic Diseases Syracuse N Y*

The congenital form of human toxoplasmosis the first from which *Toxoplasma* was isolated may be the most informative source of knowledge concerning the interrelationship between this parasite and man It is from this view point rather than as a pure clinical problem, that the subject will be discussed at this time The infectiousness of *Toxoplasma* for humans was proved when Wolf Cowen, and Paige[1] isolated the parasite from a 31 day old infant whose illness first had been noted on the third day of life The presence of chorioretinitis and diffuse encephalomyelitis was corroborated at autopsy and *Toxoplasma* like organisms were seen in the histological sections Most importantly, *Toxoplasma* was demonstrated in mice and rabbits who succumbed following injection with suspensions of the infant s brain and spinal cord Here then was evidence not only that *Toxoplasma* could produce fatal congenital human disease but, also that it could give rise to inapparent infections in human adults (for the mother though well presumably had been the source of the infection) This was proof too that chorioretinitis and encephalomyelitis could result from infection with *Toxoplasma* Subsequently, it was demonstrated that microcephaly and macrocephaly cerebral calcifications convulsive disorders and mental retardation could accompany or follow congenital infections and that such infections were manifested either *in utero* or at varying periods following birth

Further progress was slow (and in some instances even misleading) until 1948 when new (or improved) precise and informative techniques the dye skin and complement fixation tests became available These served to stimulate world wide interest in the parasite and the disabilities that it may produce so that much new information has been acquired during the past seven years Because limitations of time and space do not permit a complete discussion of all or much of this newer knowledge this presentation will be limited to a discussion of the information derived from the preliminary analysis of 187 cases that we have accepted as representing instances of congenital toxoplasmosis We have nothing to contribute in a positive sense to the question of whether congenital toxoplasmosis is ever inapparent

Among 176 of these cases 119 (68 per cent) were 4 years of age or less (60 1 year or less) 38 (22 per cent) were from 5 to 9 years and 19 were in the age group of 10 to 19 years There seemed to be no good reason to exclude these older cases

The dye test antibody patterns of many of the mothers and their offspring are illustrated in FIGURES 1 and 2 As with any serological measurement there are individual variations but antibodies tend to persist at significant levels for many years No serologically similar group of mat[illegible] has ever been

* Th[illegible] study was supported by [illegible] from the Nat[illegible] I[illegible] of H[illegible]lth B[illegible]th[illegible] Md

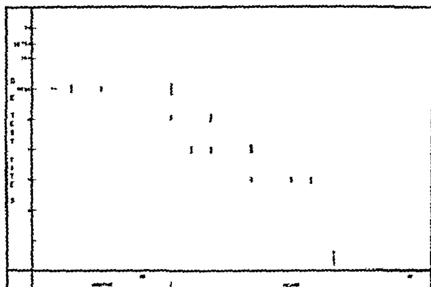


FIG. 1. Toxoplasma dye test titers of 11 sera from 11 mothers by interval after birth of infants with congenital toxoplasmosis.

studied by us or in so far as we are aware by anyone else. Despite this, we have no reason to assume that antibodies will not persist in the same fashion in men. One could conclude, though, that these high titers encountered in inapparent infections of pregnant females are due in part to continued stimulation by antigenic material released from the infected fetus.

The differences between the antibody pattern of passively and actively immunized infants are illustrated in TABLE 1. These are typical of many such experiences; they support the statement[3] that titers of 1:256 in an infant four *or more months of age generally are due to infection and not to passive transfer*. There are some who believe that when such a titer is obtained in a maternal serum it means that the mother recently has given birth to a congenitally infected infant. We do not believe that such a conclusion is warranted except under very carefully defined circumstances.

We have assumed (this is not unique) that the congenital disease results when a nonimmune pregnant woman happens to acquire an inapparent infection. *The resultant parasitemia permits Toxoplasma to set up a nidus in the* placenta from which infection of the fetus follows. We have seen such a lesion in only one placenta, all that has been available for our study. The great majority of the mothers of our cases has been between 18 and 29 years of age, the remainder being between 30 and 39 years. This young maternal age would appear to be related to an age group of increased susceptibility to the disease *in question*. These data could be interpreted also as opposing the concept that infected infants often are born some years after the mother has acquired her initial infection.

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*By* Harry A Feldman and Louise T Miller

*Department of Public Health and Preventive Medicine State University of New York Upstate Medical Center at Syracuse N Y and the Kilian Research Laboratory Wieting Johnson Hospital for Rheumatic Diseases Syracuse N Y*

The congenital form of human toxoplasmosis the first from which *Toxoplasma* was isolated may be the most informative source of knowledge concerning the interrelationship between this parasite and man It is from this view point rather than as a pure clinical problem, that the subject will be discussed at this time The infectiousness of *Toxoplasma* for humans was proved when Wolf Cowen, and Paige[1] isolated the parasite from a 31 day old infant whose illness first had been noted on the third day of life The presence of chorioretinitis and diffuse encephalomyelitis was corroborated at autopsy and *Toxoplasma* like organisms were seen in the histological sections Most importantly *Toxoplasma* was demonstrated in mice and rabbits, who succumbed following injection with suspensions of the infant s brain and spinal cord Here then was evidence not only that *Toxoplasma* could produce fatal congenital human disease but also that it could give rise to inapparent infections in human adults (for the mother though well presumably had been the source of the infection) This was proof too that chorioretinitis and encephalomyelitis could result from infection with *Toxoplasma* Subsequently, it was demonstrated that microcephaly and macrocephaly cerebral calcifications convulsive disorders and mental retardation could accompany or follow congenital infections and that such infections were manifested either *in utero* or at varying periods following birth

Further progress was slow (and in some instances even misleading) until 1948 when new (or improved) precise and informative techniques the dye skin and complement fixation tests became available These served to stimulate world wide interest in the parasite and the disabilities that it may produce so that much new information has been acquired during the past seven years Because limitations of time and space do not permit a complete discussion of all or much of this newer knowledge this presentation will be limited to a discussion of the information derived from the preliminary analysis of 187 cases that we have accepted as representing instances of congenital toxoplasmosis We have nothing to contribute in a positive sense to the question of whether congenital toxoplasmosis is ever inapparent

Among 1/6 of these cases 119 (68 per cent) were 4 years of age or less (60 1 year or less) 38 (22 per cent) were from 5 to 9 years and 19 were in the age group of 10 to 19 years There seemed to be no good reason to exclude these older cases

The dye test antibody patterns of many of the mothers and their offspring are illustrated in FIGURES 1 and 2 As with any serological measurement there are individual variations but antibodies tend to persist at significant levels for many years No serologically similar group of males has ever been

* Th t dy w pp t d by g a t f m the N t I I t t t I H I I E th d Md

stances cannot occur. We are only indicating that they would be unusual events in our area.

Among 141 of our cases approximately one fourth were born prematurely. This is somewhat, but not greatly, in excess of the normal expectation. Some 20 per cent of the prematures died in contrast with 7 per cent of the term births. That this difference in survival is due to toxoplasmosis solely is questionable for the difference is no greater than generally would be expected between premature and term births.

Is the mother of an infant with congenital toxoplasmosis liable to produce another such baby? In other words is she likely to shed parasites into her bloodstream or maintain viable organisms in the sites of placental implantation? We analyzed 204 pregnancies in such mothers by comparing the outcome of preceding and subsequent gestations and found no differences of significance. We have yet to encounter the first instance where a given mother produced more than one congenitally infected offspring.

Among 108 cases with chorioretinitis we found that 92 per cent had lesions in both eyes. Whether the remainder actually had unilateral lesions only is questionable but the frequency of bilateral involvement tends to support the thesis that the chorioretinitis results from bloodstream seeding. If this experience can be carried over to the eye disease in acquired toxoplasmosis one might expect a significant number of these to be bilateral too.

The birth months of 108 patients who were born north of the equator were plotted and it was found that the frequency from month to month was essentially the same which indicates that the infection probably can be acquired in any month or season of the year.

Does each child have the same degree of residual damage? The answer is no again although almost all had detectable chorioretinitis and most had cerebral calcifications, mental retardation and or disturbances in head size. This does not mean that every infant or child with chorioretinitis or cerebral calcifications has congenital toxoplasmosis. In fact no more than half such cases have turned out to be due to this infection.

It has been impossible for us to detect retrospectively any illness pattern during pregnancy that suggests when the mothers might have acquired their infections. We have therefore been unable to project this information for future use. Indeed four fifths of these mothers denied having had any illness at all.

Most of the fathers whose sera were made available to us have not had detectable antibodies which argues somewhat against the probability that person-to-person transfer is commonplace or for that matter important.

To sum up we believe that this analysis of a significant number of cases of congenital toxoplasmosis indicates that the disease occurs as an accidental complication of an inapparent primary toxoplasma infection of a pregnant female because such infections may be accompanied by parasitemia. In so far as we are aware all such infants suffer some residual damage but most survive. The infection may be acquired in any season of the year and human-to-human transfer is not commonplace. This complication is not repeated in

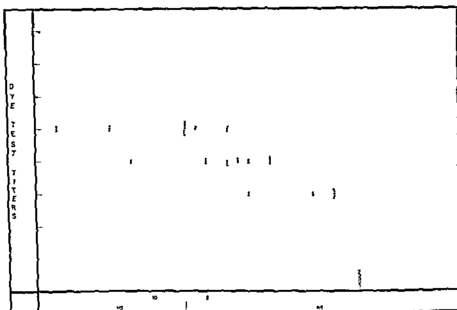



FIGURE Toxoplasma dye test titers of 119 a by int rs l ne birth of 110 off p ing w th g tal t oplasm is

**TABLE 1**

**TOXOPLASMA DYE TEST ANTIBODY PATTERNS ILLUSTRATING PASSIVE TRANSFER AND CONGENITAL DISEASE**

| St at | D y t pa tum | Dye test t te | |
|---|---|---|---|
| | | Mat rn l | Infa t |
| Pa ive transfer | 0 | 256 | 128 |
| | 42 | — | 128 |
| | 90 | — | 16 |
| | 132 | 256 | 4 |
| Congenital toxopla mosis | 50 | 1024 | 512 |
| | 127 | 1024 | 1024 |

The sex of the offspring plays no role in this disease since 53 per cent of our cases were males and 47 per cent were females

We have been unable to relate the time of acquisition of infection with the end result Our hypothesis has been that the earlier in pregnancy the infection is acquired the more catastrophic the effect i e if early a spontaneous abortion would result if in the second trimester a stillbirth or a premature if in the last trimester a term birth with lesser or greater degrees of residual damage We have not encountered an instance where we could demonstrate toxoplasmosis to be the cause of spontaneou abortion nor have we been able to detect infection as evidenced by a change in antibody status in about 600 pregnant women who were followed to term This is not to say that such in

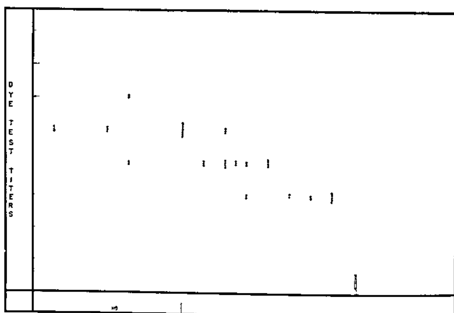



FIGURE 2. Toxoplasma dye test titer of 119 sera by interval ... birth of 110 offspring ... congenital toxoplasmosis

TABLE 1
TOXOPLASMA DYE TEST ANTIBODY PATTERNS ILLUSTRATING PASSIVE TRANSFER AND CONGENITAL DISEASE

| Situation | Days postpartum | Dye test titers | |
|---|---|---|---|
| | | Maternal | Infant |
| Passive transfer | 0 | 256 | 128 |
| | 42 | — | 128 |
| | 90 | — | 16 |
| | 132 | 256 | 4 |
| Congenital toxoplasmosis | 50 | 1024 | 512 |
| | 127 | 1024 | 1024 |

The sex of the offspring plays no role in this disease since 53 per cent of our cases were males and 47 per cent were females.

We have been unable to relate the time of acquisition of infection with the end result. Our hypothesis has been that the earlier in pregnancy the infection is acquired the more catastrophic the effect, *i.e.*, if early, a spontaneous abortion would result; if in the second trimester, a stillbirth or a premature; if in the last trimester, a term birth with lesser or greater degrees of residual damage. We have not encountered an instance where we could demonstrate toxoplasmosis to be the cause of spontaneous abortion, nor have we been able to detect infection as evidenced by a change in antibody status in about 600 pregnant women who were followed to term. This is not to say that such in-

# TOXOPLASMOSIS ACQUISITA LYMPHONODOSA: CLINICAL AND PATHOLOGICAL ASPECTS*

By J. Chr. Siim
*The State Serum Institute, Copenhagen, Denmark*

In 1937–1939 Wolf, Cowen, and Paige published details of the first verified cases of congenital toxoplasmosis and gave a clinical description of this now classical disease entity.

The introduction of reliable quantitative reactions for the serological diagnosis—the Sabin-Feldman dye test and the complement fixation (CF) test (Warren and Russ; Sabin)—aroused much interest among clinicians from almost all fields of medicine. Papers appeared subsequently in which toxoplasmosis was diagnosed in both children and adults suffering from a wide variety of the most varying disease conditions. In some of these reports, however, the diagnosis was based upon microscopic examination and upon seroreactions carried out on single blood samples only, using many different techniques. It should be emphasized that it is unsafe to diagnose toxoplasmosis on morphological grounds alone, especially when dealing with fixed tissue preparations. In addition, it is also difficult to interpret the results of serological examinations because (1) dye test antibodies frequently occur among apparently healthy persons, (2) the seroreactions remain positive for a long period after the acute stage of the disease, and (3) antibody formation also appears after inapparent infections. The demonstration of increased titers alone in single serological specimens can therefore by no means be taken as proof that cases of uncertain etiology are of toxoplasmic origin. This can be confirmed only by the demonstration of rising titers in a new test simultaneously with the development of clinical features or by the isolation of *Toxoplasma*. Furthermore, the use of nonstandardized diagnostic methods and antigens makes comparison of results from different places almost impossible.

Few laboratories seem to perform routine diagnostic isolation experiments and difficulties arise in clinical practice in procuring specimens for inoculation into experimental animals. Therefore, although it cannot be excluded *a priori* that some of the patients recorded might have been infected with *Toxoplasma*, in many of the cases it has not been feasible to perform isolation experiments, thus establishing the final academic proof of a correlation between the actual disease condition and a toxoplasmic infection by demonstration of the parasite responsible.

The following account includes only clinical entities in which the occurrence of an acquired infection with *Toxoplasma* has been established by: (1) isolation of *Toxoplasma* in well controlled animal experiments from patients with characteristic clinical, histological, and serological findings; (2) demonstration of a significant rise in titer in the acute stage of the disease simultaneously with the development of characteristic signs and symptoms.

Using these criteria, it is possible to divide acquired infectious disorders caused by *Toxoplasma gondii* into four main manifestations (TABLE 1), charac-

* Aided by a grant from The King Christian X Foundation, Copenhagen, Denmark.

subsequent pregnancies and it affects more than one offspring only if the reference pregnancy results in multiple births While most infants with congenital toxoplasmosis have residual chorioretinitis many instances of this problem are due to other causes

*References*

1 WOLF A D COWEN & B H PAIGE 1939 Science 89 226
2 SABIN A B H EICHENWALD H A FELDMAN & L JACOBS 1952 J Am Med Assoc 150 1063

both encephalitis and myocarditis were present for more than five months survived On post mortem examination cell infiltrations and necrotic foci in heart lungs and central nervous system are present with *Toxoplasma* like structures in the myocardium in all the cases examined

*Toxoplasmosis Acquisita Cerebrospinalis*

The first case was reported by Sabin in 1941 A six year-old boy showed fever delirium generalized convulsions lymphadenopathy and a mononuclear pleocytosis After 29 days the patient died On post mortem examination a meningoencephalitis was found and the RH strain of *Toxoplasma* was isolated from the brain

This manifestation of acquired toxoplasmosis seems to occur but seldom In Denmark only one other similar case has hitherto been observed that of a three year-old girl in the United States Feldman (1953) has reported on encephalitis and a rash in a six year old boy in North Africa Giroud *et al* (1953) have isolated strains from three adults and in Germany Frenkel and Horst reported a case in 1952

*Toxoplasmosis Acquisita Ophthalmica*

The first verified case of ocular toxoplasmosis has recently been reported by Jacobs Fair and Bickerton (1954) In a 30-year-old man with a chronic chorioretinitis *Toxoplasma* were isolated from an enucleated eye

*Toxoplasmosis Acquisita Lymphonodosa*

*Clinical findings* While the above mentioned manifestations of the acquired toxoplasmic infection seem to occur rather seldom toxoplasmosis with a lymphadenopathy as the main sign is relatively frequently diagnosed The first Danish case was reported five years ago at the Sixth International Congress of Pediatrics at Zurich Switzerland 1950 (Siim 1950) and at the same time a Swedish paper on the subject was published by Gard and Magnusson (1950 and 1951) Since then toxoplasmic lymphadenopathy has been reported from several countries in 1951 and 1952 from Denmark by Siim from Sweden by Gard Magnusson Wising Zeipel and Linder in 1953 from Denmark by Bang Rasmussen and Siim from France by Faure from Sweden by Jacobsson and from the United States by Armstrong and MacMurray and by Stanton and Pinkerton in 1954 from England by Skipper Beverley and Beattie and by Cathie from France by Lelong Desmonts Vinh Nezelof Satge and Couvreur and by Sedallian Garin and Faure and finally in 1955 from Denmark by Siim and from Finland by Gronroos Further cases have been observed in France by Desmonts in Germany by Fiekarski and in Sweden by Huldt

Based upon clinical observations of our cases in which *Toxoplasma* have been isolated it is possible to divide the lymphadenopathic form into three subgroups (1) febrile lymphadenitis (2) a nonfebrile form and (3) a subclinical form

TABLE 1

VARIOUS CLINICAL MANIFESTATIONS OF HUMAN TOXOPLASMOSIS THE EXISTENCE OF WHICH HAS BEEN VERIFIED BY POSITIVE ISOLATION EXPERIMENTS

| Congenita | Acquisita | | | |
|---|---|---|---|---|
| | Lymphonodosa | Exanthematica | Cerebrospinalis | Ophthalmica |
| | Febrile Afebrile Subclinical (1) Lymphadenopathy (2) Relative lymphocytosis atypical morphology Paul Bunnell negative (3) Lymph node biopsy characteristic histology isolation of toxoplasma (4) No complications (or very seldom) | Typhuslike exanthema Atypical pneumonia Myocarditis Meningoencephalitis Often fatal | Encephalitis (rash) | Chorioretinitis |

terized by (1) lymphadenopathy (2) typhuslike exanthema (3) meningoencephalitis (4) chorioretinitis

These conditions cannot of course be fulfilled in clinical practice on a broader scale It seems important however to emphasize that various forms of acquired toxoplasmosis exist especially considering that the frequent occurrence of antibodies in the population has caused a certain doubt among clinicians regarding the value of the diagnostic methods available

*Toxoplasmosis Acquisita Exanthematica*

Five cases of exanthematic acquired toxoplasmosis have been reported with positive isolation experiments (Pinkerton and Henderson 1941 Kass Andrus Adams Turner and Feldman 1952 Sexton Eyles and Dillman 1953 Giroud and Grjebine 1951 1953) In an additional case a significant rise in the antibody titer was demonstrated during the acute phase of the disease (Strom, 1951)

The clinical picture of this generalized infection is characterized by a typhus like illness the onset of which is often acute with chills and temperature of about 40 C in some cases preceded by fatigue and malaise of a few days to a week s duration The typical sign is a red nonhemorrhagic maculopapular exanthema that involves the whole body except for the scalp the palms and the soles of the feet The rash appears during the first week at the earliest on the fourth day and disappears one to two weeks later Dry cough and pulmonary changes simulating atypical penumonia are usually found both early and later during the course of the disease but might be completely absent Symptoms or signs of myocarditis and meningoencephalitis often complicate the disease lymphadenopathy might be found but enlargement of the spleen has not been demonstrated Most cases have been fatal only one patient in whom signs of

Because of the demonstration of a relative lymphocytosis together with a negative Paul Bunnell test, seroreactions for toxoplasmosis were carried out on November 3 with positive result (dye test 1:1250). The reactions were negative on October 17 (FIGURE 1). On renewed clinical examination on November 21 slightly tender lymph nodes, the size of hazelnut kernels, were revealed in both axillae and in the left inguen. The nodes were rather firm, discrete, with no attachment to the underlying tissue, and the covering skin was unaffected. Fifty-one days after onset a lymph node was excised from the right axilla and a strain of *Toxoplasma* was isolated.

*Course.* The temperature was about 39° C. from the 16th to the 20th of October and then gradually decreased. From November 23 the temperature was normal, having been elevated for 43 days. The patient was discharged on December 5 after having been in the hospital for 50 days. No complications were observed except for a febrile retinopathy.

*On follow up* six months after commencement of the disease the patient was fit and well and had done light work. The indolent lymphadenopathy was still present, the dye test was 1:6750 and the C.F. test 1:32(64).

*Treatment.* No treatment was instituted except for confinement to bed for 38 days and injections of procaine penicillin, 300,000 units, were administered for the first five days without any effect on temperature.

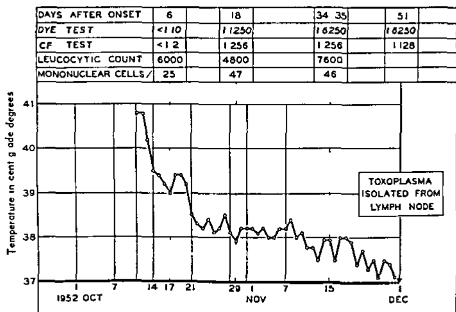
*Laboratory findings.* Hemoglobin value on October 23, November 3 and 25: 95, 93 and 99 per cent, respectively. Erythrocyte count and color index on October 23: 4.75 million and 1.07. Sedimentation rates on October 20, November 13 and December 4 were 39, 58 and 34 mm. per hour (Westergren). Leucocyte counts on October 17 and 29 and on November 14: 6,000, 4,800 and 7,600, respectively. The differential counts on these dates were: unsegmented 9 per cent, 6 per cent and 7 per cent; segmented 65 per cent, 42 per cent and 35 per cent; eosinophils 0 per cent, 3 per cent and 12 per cent; large lymphocytes 9 per cent, 27 per cent and 29 per cent; small lymphocytes 14 per cent, 18 per cent and 14 per cent; monocytes 2 per cent, 2 per cent and 3 per cent; plasma cells 1, 2 and 0 per cent. The large lymphocytes were slightly atypical. Thymol turbidity test on November 26 and December 1: 0.10 (negative) and 0.23 (positive). The urine contained albumin on October 17 and 27 (0.1 per thousand) without positive microscopic findings. Serum protein total 6.50 per cent. Albumin/globulin 0.83. Spinal fluid on October 16 and 19 normal (0 and 4/3 cells per cubic millimeter). Wassermann reaction negative. Agglutination lysis test for leptospirosis and Widal's reaction were negative. Mantoux test negative. Paul Bunnell test on October 17 and 29 and on November 18 negative (1:8). Antistreptolysin titer on October 25 and November 26: 43 and 50 (normal).

*X-ray of chest:* no enlargement of hilus shadows.

*X-ray of skull:* no intracerebral calcifications.

*Electrocardiogram:* normal.

*Ophthalmoscopy* on November 26 showed retinal exudates and very fine hemorrhages on the left eye (febrile retinopathy?). Five months later the examination was normal.




Fi r 1 F b l f m of t pl m q is t l mpho od sa 39 year-old man (Case No 7) Isola t f To pl m f m llary lymph od sed 51 d y ft set

*Febrile Lymphadenitis*

The onset may be acute with chills and fever of 38 to 40° C, but in some cases the commencement is gradual The temperature lasts for two to four weeks or even longer and then decreases by lysis

The following two case records are representative of this manifestation

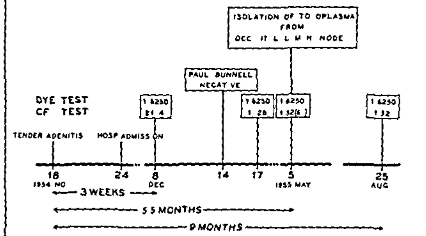
*Case No 7* (TABLE 7 FIGURE 1)

*History* (Case No 6022 of the Blegdamshospitalet or Epidemic Hospital of Copenhagen) A 39 year old bricklayer previously in good health was suddenly taken ill on October 11 1952 with chills and temperature of 40 8° C No symptoms apart from moderate headache tenderness in the back and slight angina

As the temperature remained elevated the patient was admitted to hospital on October 16 with continued fever and albuminuria

On admission the general condition was unaffected temperature 39 2 C pulse rate 80 Except for some redness of the fauces and tender swelling of the left angular lymph node there were no other associated signs and particularly no exanthema no respiratory cardiac muscular or neurological signs no lymphadenopathy or enlargement of liver or spleen

*Diagnosis* The preliminary diagnosis was acute tonsillitis infectious mononucleosis? continued fever




Fi r e 2 F l le f rm f t plasm si q ta l m l d sa in 20-y -old woman (C No 10) Isolat n of To plasma l m an oc p tal lymph d is d 5 5 m ths ft nset

*On admission* the temperature was 3 C pulse rate 2 general condition unaffected Examination showed nothing abnormal except for redness of the fauces and lymphadenopathy There was swelling of the occipital preauricular and cervical lymph nodes These were the size of hazelnut kernels were smooth firm uniform and freely mobile but tender No lymph nodes could be demonstrated with certainty in the axilla and inguen There were no other signs especially no respiratory cardiac muscular or neurological signs no enlargement of the liver or spleen and no exanthema The skin was normal and the teeth in good condition

*Diagnosis* The preliminary diagnosis was acute pharyngitis and adenitis Because of the demonstration of lymphocytosis with atypical cells accompanied by a negative Paul Bunnell test on December 8 seroreactions for toxoplasmosis were carried out with positive result (dye test 1 6250)

*Course* The temperature became normal in three days In spite of this the lymphadenopathy increased *On December 22 tender lymph nodes were* found in both axillae and inguina

On discharge on December 20 *i e* approximately one month after commencement of the disease there were no complaints except for pronounced fatigue

*On follow up* on February 23 1955 the lymph nodes that were previously affected showed no change and in addition new very tender nodes were found along the left border of the trapezius

Five and one half months after the recognition of lymphadenopathy the pa

*Examination for toxoplasmosis* On the 3rd, 15th and 25th of November the 1st and 29th of December 1952, and the 20th of April 1953, the dye tests were 1 1250, 1 6250 1 6250, 1 6250 1 6250 1 6250 The CF tests on the same dates no serum 1 256 1 256 ≧ 1 128, ≧ 1 128, 1 32(64)

The blood sample, tested for heterophile antibody on October 17, did not contain *Toxoplasma* antibody (dye test < 1 10, CF test < 1 2)

*Isolation of* Toxoplasma On December 1 1952, a lymph node was excised from the right axilla and a strain of *Toxoplasma* was isolated by inoculation intraperitoneally in clean mice (TABLES 7 and 8)

*Treatment* Procaine penicillin 300 000 units for five days without any effect on temperature

*Source of infection* uncertain The patient had often eaten raw meat and raw eggs, the last time about one month before the onset of the illness He had been stung by gnats and it had been observed that birds had been eating of the family s food stored on a balcony in the open air

*Summary* A 39 year-old bricklayer was suddenly taken ill with chills fever of 40 8° C and moderate headache malaise and slight sore throat On admission to the hospital five days later the general condition was unaffected On clinical examination there was only some redness of the fauces and a tender angular lymph node on the left side The preliminary diagnosis was tonsillitis glandular fever and continued fever As a relative lymphocytosis and a negative reaction to a Paul Bunnell test was found seroreactions for toxoplasmosis were performed on the 24th day of illness with positive results (dye test 1 1250) Renewed examination revealed moderate axillary and inguinal lymphadenopathy

In this case a significant rise in titer was observed simultaneously with the development of the lymph node swelling and the lymphocytosis The diagnosis was finally verified by isolation of *Toxoplasma* from a lymph node biopsy 51 days after onset of the disease

The course was prolonged and uncomplicated except for a febrile retinopathy The temperature remained elevated for 43 days and the patient complained of fatigue for about four months On follow up six months after commencement of illness the patient was fit and well but lymphadenopathy was still present the dye test being 1 6250 and the CF test 1 32(64)

*Case No* 10 (TABLE 7 FIGURE 2)

*History* (Case No 2/16/54 of Department F of The Copenhagen County Hospital Copenhagen) This was a 20 year old probationary nurse previously healthy apart from myxoedema diagnosed in 1953 angina tonsillaris in August 1954 (differential blood counts normal) considerable anemia (66 per cent) in September 1954 and febrile catarrhalia from October 22 to 28 1954 Without any previous symptoms particularly no chills fever or angina the patient discovered on November 18 and 19 1954 a tender swelling in front of the left ear Since this increased in size during the subsequent days and as a similar swelling occurred in front of the right ear she was admitted to hospital on November 24 1954 with a diagnosis of adenitis The patient felt unwell and feverish but otherwise there had been no symptoms

On admission to hospital for adenitis six days later the general condition was unaffected except for pronounced fatigue and the temperature was only 37.4 C for about three days The clinical examination showed nothing abnormal except for redness of the fauces and swollen tender lymph nodes in front of the ears in the occipital region and on the neck The laboratory examination showed increased sedimentation rate relative lymphocytosis positive thymol turbidity test and negative Paul Bunnell test Seroreactions for toxoplasmosis were performed with positive results (dye test 1 6250 CF test 1 178) After three weeks confinement to bed the patient was discharged about one month after commencement of the disease There were no complications but pronounced fatigue The lymphadenopathy was still present five and one half months after the onset and at that time *Toxoplasma* were isolated from a lymph node removed by biopsy from the left occipital region

*Case No* 8 (TABLES 6 and 7)

A *nonfebrile form* is also seen in which the enlarged lymph nodes are often discovered by the patients themselves or by their mothers as in this case of a 12 year-old schoolboy in whom a hard cervical lymphadenopathy was observed

*History* (The Finsen Institute Pediatric Outpatient Department Case No 76566 and Blegdamshospitalet Epidemic Hospital of Copenhagen Case No 1131/41 A 12 year-old boy previously in good health observed by accident about October 14 a swelling (not tender) on both sides of the neck There were no other symptoms no temperature and the general condition was completely unaffected As the swelling persisted the mother consulted the physician a week later because of fear of a malignant condition

*On examination* the general condition was unaffected and the temperature normal Except for lymph nodes the size of beans on both sides of the neck not tender there were no other signs particularly no exanthema no respiratory cardiac muscular or neurological signs there was no generalized lymphadenopathy or enlargement of the spleen or liver

*Diagnosis* The preliminary diagnosis was streptococcal infection infectious mononucleosis ( ) toxoplasmosis (?) and cervical adenitis

Because of the demonstration of lymphadenopathy and relative lymphocytes seroreactions for toxoplasmosis were carried out on the initiative of the practitioner A Kent on November 3 with positive results (dye test 1 6250 CF test 1 64(128)) Seven and one half weeks after commencement of the disease a lymph node was removed from the right side of the neck and *Toxoplasma* were isolated by intraperitoneal inoculation into mice

*The course* was benign with no neurological complications or eye symptoms The swelling of the lymph nodes was still present seven months after onset

Twenty two months later the patient was admitted to the Epidemic Hospital Copenhagen with a clinically typical infectious mononucleosis There was generalized lymphadenopathy the Paul Bunnell reaction was positive (1 128 absorption *ad modum* Davidsohn typical) leucocytic count 18 000 differential count polynuclear cells 7 per cent eosinophils 1 per cent large lymphocytes 90 per cent small lymphocytes 1 per cent and monocytes 1 per

tient still felt tired but was able to carry out her work. The tender enlarged lymph nodes still persisted. A lymph node was removed from the left occipital region and *Toxoplasma* were isolated.

On follow up about three and one half months later, the patient had been fit and well for one month. The lymph node swelling was decreasing but still tender. The dye test was 1:6250 and the CF test 1:32.

*Treatment.* Confinement to bed for three weeks and penicillin injections for 12 days (dipenicillin 400 000 units, for 4 days and G penicillin 1 million units twice a day for 8 days).

*Source of infection.* The family was healthy and there were no similar cases at the patient's place of employment. The patient had eaten raw eggs but no uncooked meat or milk.

*Laboratory findings.* Hemoglobin value 95 to 89 per cent. Erythrocytes counts 4.8 million. Index color 0.88 and 1.01. Sedimentation rate on November 26, December 6 1954 and May 26 1955: 30, 21 and 27 mm per hour (Westergren). Leucocytic counts on November 13 and 26, December 7 and 14, January 1 and May 26 1955: 3 600, 5 400, 7 700, 5 600, 5 900, 7 800. The differential counts on the same dates were: segmented 53 per cent, 39 per cent, 42 per cent, 45 per cent, 33 per cent, 46 per cent; unsegmented 1 per cent, 1 per cent, 3 per cent, 1 per cent, 1 per cent, 1 per cent; eosinophils 4 per cent, 0 per cent, 1 per cent, 2 per cent, 2 per cent, 0 per cent; basophils 0 per cent, 0 per cent, 0 per cent, 1 per cent, 2 per cent, 0 per cent; monocytes 3 per cent, 0 per cent, 2 per cent, 4 per cent, 6 per cent, 4 per cent; lymphocytes 39 per cent, 60 per cent, 52 per cent, 47 per cent, 58 per cent, 49 per cent; the large lymphocytes were atypical similar to cells seen in infectious mononucleosis. Thymol turbidity test on December 9 and June 13: 0.52 (positive) and 0.13. Icterus index (Meulengracht) 6. Urobilin negative. Urobilinogen negative. Serum bilirubin 0.48 mg per cent. Takata Ara test negative. Antistreptolysin titer on November 26 and December 10: 220 and 200. Wassermann reaction negative. Paul Bunnell test on December 6 and 14 negative (<1:8). Throat culture: no growth of hemolytic streptococci.

*X rays of the chest:* no enlargement of the hilus shadows.

*Ophthalmoscopy:* Normal.

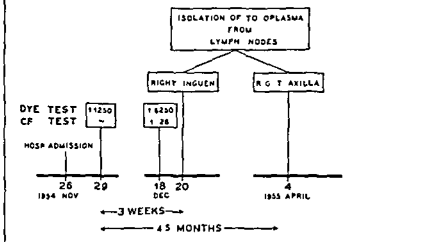
*Electrocardiogram:* Normal.

*Examination for toxoplasmosis.* On December 8 and 17, January 19, February 23, March 31, June 13 and August 28 the dye test titers were 1:6250, 1:1250(6250), 1:6250, 1:6250, 1:1250, 1:6250 and 1:6250. The CF titers on the same dates were ≧1:4, 1:128, 1:128, 1:64, 1:64, 1:32(64) and 1:32.

*Isolation of* Toxoplasma. On May 5 1955 a lymph node was removed from the left occipital region and *Toxoplasma* were isolated by intraperitoneal inoculation into mice (TABLES 7 and 8).

*Summary.* A 20 year old woman had previously been in good health except for myxoedema (diagnosed 1953), angina tonsillaris in August 1954, considerable anemia in September 1954 and febrile catarrhalia from October 22 to 28 1954.

On November 18 and 19 the patient discovered a tender lymph node in front of the left ear and a few days later a similar one on the right side.




F g e 5 S bel nical f rm f t plasm is a quisit lymph od sa in a 12 year-old g l (Cas \ 9) *T septs* was isolated from guinal lymph nod biopsy d 4 5 mo th lat also f m a ll ry nod

apparently somatically healthy was admitted to hospital for observation for an oligophrenia of about nine years' duration

*History* (Case No 1169 54, The Children s Hospital University of Copenhagen) A 12 year-old girl previously in good health was admitted to hospital for closer observation for oligophrenia which started at the age of about three years without previous acute infection or trauma of the head Except for minor catarrhal disorders the patient has not been ill during recent years In particular she had had no attacks of fever

*On admission* on November 26 1954 the general condition was unaffected there were no complaints and the temperature was normal On neck body and proximal parts of the thighs the remains of exanthema (pityriasis rosea like) were seen Except for a left sided strabismus no signs were present particularly no respiratory cardiac muscular or neurological signs There was no enlargement of liver or spleen

*Diagnosis* The preliminary diagnosis was oligophrenia and on routine examination for toxoplasmosis (carried out at that time on all neurological and psychiatric patients) positive seroreactions were revealed At the same time on renewed examination lymph nodes the size of cherries were found in both axillae and lymph nodes the size of peas in the inguen There was no adenitis on the neck and no swelling of the cubital lymph nodes The lymph nodes were smooth hard mobile and were covered by normal skin

cent The thymol turbidity test was positive (0 54), dye test, 1 50(250), CF test unreadable

*Follow up* Three years after the onset the patient was fit and well there was no lymphadenopathy or enlargement of the spleen The dye test was 1 50(250) the CF test 1 8

*Treatment* Except for Finsen light, no therapy was instituted

*Laboratory examination* On October 30 the hemoglobin value was 99 per cent sedimentation rate, 10 mm per hour (Westergren) Leukocytes counts on October 30 and November 13 5 000 and 5 400 Differential counts on these dates neutrophil leukocytes 18 and 30 per cent, eosinophils 6 and 4 5 per cent basophils, 2 and 0 per cent, lymphocytes, 70 and 58 5 per cent monocytes 4 and 7 per cent The large lymphocytes were atypical Antistreptolysin titers on November 14 January 19 and May 18 400 140 and 250 Throat culture no growth of hemolytic streptococci Wassermann reaction negative Paul Bunnell test negative (<1 8) on November 3 and May 22

*Examination for toxoplasmosis* On November 3 December 6 1952, January 19 1953, May 18 August 27, 1954 September 5, and on September 25, 1955, the dye test readings were 1 6250, 1 6250 1 6250 1 1250 1 50(250) 1 50, 1 50(250) The CF test readings on the same dates were 1 64(128), 1 128, 1 64, 1 8 not enough serum unreadable and 1 8

*Isolation of* Toxoplasma On December 6 1952 a lymph node was removed from the right side of the neck and a *Toxoplasma* strain was isolated by intraperitoneal injection into clean mice (TABLES 7 and 8)

*Source of infection* uncertain The patient often ate raw meat and raw eggs The family the members of which were healthy had a cat which had not been ill (serological tests could not be performed)

*Summary* A hard cervical lymphadenopathy was observed by accident by a 12 year old schoolboy The general condition was unaffected the temperature normal and there were no other symptoms or signs One week later, the physician was consulted because of the fear of malignancy Swollen cervical lymph nodes and a relative lymphocytosis (74 per cent) were found The preliminary diagnosis was streptococcosis adenitis infectious mononucleosis (?), toxoplasmosis (?)

The sereoreactions for toxoplasmosis were strongly positive [dye test 1 6250 CF test 1 64(128)] A lymph node was removed from the right side of the neck about seven and one half weeks after onset of the disease and *Toxoplasma* were isolated from this biopsy by inoculation into mice

The course was uncomplicated About 22 months later the patient developed a clinically typical infectious mononucleosis with characteristic hematological and serological findings The dye test was 1 50

At follow up examination three years after onset the patient was fit and well There were no palpable lymph nodes The dye test was 1 50(250) and the CF test 1 8

*Case No 9* (TABLE 7 FIGURE 3)

*A subclinical form* in which the lymphadenopathy is found in the course of routine examination or among contacts of verified cases A 12 year old girl

### *Clinical Course*

The State Serum Institute receives blood samples from the whole of Denmark and because of the small size of the country it has been possible to examine the patients personally and to carry out follow up examinations in close cooperation with the hospitals and general practitioners

Generally the disease runs a benign course most of the patients making complete recovery The enlargement of the lymph nodes however persists for 6 to 12 months or even longer In some of the cases convalescence is accompanied by pronounced fatigue over a period of months perhaps because the muscles have been affected as part of a generalized infection

No complications from the central nervous system or heart have been observed In only one of about 100 cases a chorioretinitis was seen the etiology of which could not be ascertained In a three year old girl however a lymphosarcoma developed later Malignant lymphadenopathy has been observed in one other patient with acquired toxoplasmosis whether this is purely coincidental will be the subject of further investigation

### *Serological Findings* (TABLE 2)

The seroreactions become positive within about a month and reach titers of 1 1000 to 10000 in the dye test and 1 50 to 100 in the CF test Valuable information can be obtained when both reactions are carried out simultaneously because the CF test becomes positive later than the dye test and becomes negative again earlier than the dye test The combination of a positive dye test and a negative CF reaction will therefore be found both in the acute stages of the infection and also during convalescence

With regard to the specificity of the seroreaction at the present moment maximum positive titers in both reactions can be considered specific because previously all verified cases of both congenital and acquired toxoplasmosis have been accompanied by such positive reactions On the other hand increased titers have never been found regularly in any other disease condition of well-defined etiology The problem is still open to investigation however

TABLE 2

SEROLOGICAL FINDINGS IN 10 CASES OF TOXOPLASMOSIS ACQUISITA LYMPHONODOSA

| Case | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Dye test titer | 1 6250 | 1 6250 | 1 6250 | 1 6250 | 1 1250 | 1 250<br>1 1250<br>(6250) | <1 10→<br>1 6250 | 1 6250 | 1 6250 | 1 6250 |
| CF Titer | 1 64 | 1 128 | 1 32 | <1 16→<br>1 32 | 1 32 | ≥1 4→<br>1 64 | <1 2→<br>1 256 | 1 128 | 1 128 | 1 128 |
| Paul Bunnell test | Neg<br><1 8 | Neg<br><1 8 | Neg<br><1 8 | Neg<br><1 8 | Neg<br><1 8 | Neg<br><1 8 | Neg<br>1 8 | Neg<br><1 8 | Neg<br>1 16 | Neg<br><1 8 |
| Wassermann | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |

In order to confirm the serological diagnosis a lymph node in the right inguen was excised on December 20 and four months later a lymph node was also removed from the right axilla In both cases *Toxoplasma* were isolated by injecting a lymph node suspension intraperitoneally into mice

*Laboratory findings* Hemoglobin value, 80 to 90 per cent Sedimentation rate 6 mm per hour (Westergren) On December 8 the leukocytic count was 6 440 Differential count neutrophils unsegmented 1 per cent segmented 44 per cent eosinophils 2 per cent small lymphocytes, 48 per cent monocytes 5 per cent thrombocytes 95 000 The urine contained no albumin Moro tuberculin test negative Wassermann reaction negative Paul Bunnell test on December 18, negative (1 16)

*X ray of the skull* No calcification

*X ray of the chest* Nothing abnormal

*Ophthalmoscopy* Nothing abnormal

*Electroencephalography* abnormal, with a focus in the right occipital region Spinal tap not performed

*Examination for toxoplasmosis* On November 29 and December 18 the results of the dye test were 1 1250 1 6250 The CF tests on the same dates showed no serum 1 128

*Isolation of* Toxoplasma A lymph node was removed on the 20th of December 1954 from the right inguen and on the 4th of April 1955, from the right axilla In both instances a strain of *Toxoplasma* was isolated from a suspension of the lymph nodes by intraperitoneal inoculation into clean mice

*Epidemiology* The family the members of which were healthy lived in a private house They kept no pet animals but several cats came into the garden The father stated that the patient was very keen on eating raw minced pork whenever she could get the chance

*Summary* A 12 year old girl apparently somatically healthy was admitted to the hospital for observation for an oligophrenia of about nine years duration There were no clinical findings on admission On routine examination for toxoplasmosis positive reactions were found (dye test 1 6250 CF test 1 128) Swollen but not tender lymph nodes were then found in the axillae and groins The diagnosis of toxoplasmosis was confirmed by the isolation of *Toxoplasma* from a lymph node biopsy from the right inguen and also three and one half months later from a lymph node from the right axilla

## *Physical Signs*

Except for the lymphadenopathy the clinical examination is often normal The enlarged lymph nodes are of hazelnut to walnut size They may be tender during the first weeks of the disease but later they are usually painless The nodes are firm and discrete with smooth surfaces and there is no attachment to the underlying tissue The covering skin is never affected does not itch and necrosis or formation of fistulas have never been observed The lymphadenopathy is often generalized the swollen nodes being found in the suboccipital region on the neck in the axilla and in the groin Sometimes however the lymph nodes are affected in one superficial region only Enlargement of the hilus shadows may be present The spleen is seldom palpable

TABLE 4

CONDITIONS IN WHICH SERUM TESTING FOR TOXOPLASMOSIS IS INDICATED

A Fever of unknown origin
B Lymphadenopathy (tuberculosis syphilis malignant diseases metastases)
Infectious mononucleosis (Paul Bunnell test negative)
Lymphocytosis, relative
C Encephalitis (with exanthema)
Serous meningitis
D Exanthematic diseases (typhuslike)
E Myocarditis
F Chorioretinitis

TABLE 5

DIAGNOSIS ON ADMISSION

Fever of Unknown Origin
Lymphadenopathy {Cervical, Universal, Tuberculosis}
Infectious mononucleosis
*Leucosis Hodgkin s Disease*
Metastases {Carcinoma of Breast, Carcinoma of mediastinum}
Adenophlegmone Mumps

diagnosis from cases of mild glandular fever will often present difficulties as long as the etiologic agent of this illness remains undiscovered and therefore specific diagnostic seroreactions are not available The positive *Toxoplasma* seroreactions will make a diagnosis of toxoplasmosis probable and the demonstration of pathological changes in a lymph node biopsy and positive isolation experiments will confirm that diagnosis The fundamental difference in character of the two diseases will be clear from the fact that cases have been observed with typical serological clinical and hematological features of glandular fever and that acquired toxoplasmosis with lymphadenopathy has occurred later in the same patient (Sim 1952d) Furthermore the reverse has also been *observed In a 12 year-old boy (Case No 8 TABLE 6) typical afebrile ac*

TABLE 6

AFEBRILE FORM OF TOXOPLASMOSIS ACQUISITA LYMPHONODOSA IN A 12 YEAR OLD BOY (CASE NO 8) FOLLOWED 2 YEARS LATER BY TYPICAL INFECTIOUS MONONUCLEOSIS

| | N 4 1951 | Aug 27 1954 |
|---|---|---|
| Dye test | 1 6250 | 1 50 (250) |
| CF test | 1 128 | — |
| Paul Bunnell | <1 8 | 1 128 |
| Absorption test (Davidsohn) | — | Typical |
| Lymph node | | |
| Histology | Typical | |
| Isolation of *Toxoplasma* | Positive | |

TABLE 3
EXAMINATION OF BLOOD IN 10 CASES OF TOXOPLASMOSIS ACQUISITA LYMPHONODOSA

| Case no. | Age | Hmgb % | E.S.R. mm/hour | W.B.C. | Mononuclear cells % | Atyp. cells | Thymol turb. |
|---|---|---|---|---|---|---|---|
| 1 | 17 yrs | 79 | 18 | 6200 | 74 | + | 0.18 |
| 2 | 28 yrs | 90 | 10 | 3500 | 37.5 | − | 0.14 |
| 3 | 58 yrs | 82 | 9 | 7060 | 58 | + | 0.19 |
| 4 | 3 yrs | 84 | 16 | 5300 | 72 | | — |
| 5 | 24 yrs | 95 | 8 | 8400 | 31 | − | 0.13 |
| 6 | 16 yrs | 106 | 1 | 5500 | 37.5 | − | 0.10 |
| 7 | 39 yrs | 83 | 58 | 7600 | 46 | + | 0.23 |
| 8 | 12 yrs | 95 | 10 | 5000 | 74 | + | — |
| 9 | 12 yrs | 80 | 6 | 6440 | 53 | − | — |
| 10 | 20 yrs | 89 | 36 | 7700 | 54 | + | 0.52 |

and the possibility exists that future work may reveal an antigenic relationship between *Toxoplasma* and some other organism. It should be borne in mind that positive dye tests have been reported as having occurred in animals infected with sarcosporidia and *Trichomonas* (Muhlpfort, Awad).

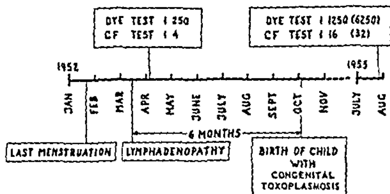
On *laboratory examination* (TABLE 3) the main clinical finding is a relative lymphocytosis with up to 50 to 70 per cent lymphocytes, the large ones having an appearance somewhat similar to the McKinlay cells found in glandular fever. The hemoglobin value is normal or only slightly reduced, and the total leukocytic and platelet counts are normal. The sedimentation rate is normal and most often only slightly increased. The thymol turbidity test may be positive, especially in the febrile cases, and it changes from negative to positive values in the acute stage of the disease (Siim, 1952a). The liver function tests are normal. The Wassermann reaction and the Paul Bunnell test are negative.

The *microscopic examination* of lymph node sections reveals a reticulum-cell hyperplasia (Siim, 1951, 1952a; Landau, 1951) and islands of large eosinophilic cells scattered over the whole of the section (Bang, 1953; Stanton and Pinkerton, 1953). The histological picture is so characteristic that when demonstrated in a lymph node biopsy it will justify a seroreaction for toxoplasmosis in order to obtain confirmation of the diagnosis.

### *Differential Diagnosis*

As the clinical features of acquired toxoplasmic infection are by no means characteristic for this disease exclusively, it is apparent that it is impossible to diagnose toxoplasmosis on the clinical picture and clinical laboratory findings alone. On the contrary, the possibility must be taken into consideration of the existence of various other disease conditions in which fever of unknown origin, lymphadenopathy, exanthema, meningoencephalitis, pneumonia, or myocarditis also occur (TABLE 4). The preliminary clinical diagnosis can be confirmed only in the laboratory by the demonstration of *Toxoplasma* antibodies in paired blood samples and by the isolation in clean laboratory mice or tissue culture of the parasite from blood, spinal fluid, lymph node, or muscle biopsies, or from specimens obtained post mortem.

The various diagnoses on admission are shown in TABLE 5. The differential




[illegible]

mentally have been successful.* Verified cases are reported from almost all parts of the world, not only among wild animals such as hares, rats and pigeons but also among pet dogs and cats and in domestic animals, pigs, cattle, sheep, rabbits and hens. The widespread occurrence among animals living in the closest contact with man is of epidemiological importance and provides rich possibilities for infection but at the same time it makes epidemiological studies extremely difficult. The following possibilities should be considered.

(1) *Toxoplasma* have been found in urine from rabbits and dogs, in feces from dogs with hemorrhagic colitis, in superficial necrotic foci in spontaneously infected pigeons (Biering-Sørensen). *Toxoplasma* pseudocysts have been demonstrated in the ovaries of naturally infected hens (Biering-Sørensen), so that it seems possible that raw eggs might be infected. *Toxoplasma* are also present in milk and in the muscles of naturally infected animals.

(2) The *mode of entry* is not known, but it is possible to infect animals experimentally via the mucous surface of the eye, the upper respiratory tracts and the vagina, and also by feeding. *Toxoplasma* have also been found to penetrate carefully shaved skin.

(3) The *mode of transmission* can only be suggested: contamination of food

[illegible]

quired toxoplasmosis with swelling of the lymph nodes on the neck was followed 21 months later by a clinically, serologically, and hematologically characteristic glandular fever. Finally, the *Toxoplasma* seroreactions have not yet been found positive in infectious mononucleosis, and in cases of verified toxoplasmosis the Paul Bunnell test has hitherto been negative.

It will also be seen from TABLE 4 that in establishing a diagnosis of toxoplasmosis, it has been possible to exclude the existence of tuberculosis, malignant diseases, *e.g.*, leukosis, Hodgkin's disease, or metastases (carcinoma of the breast, mediastinal tumor).

### *Treatment*

The sulfadiazine and Daraprim (2,4′-diamino-5-p-chlorophenyl-6-ethylpyrimidine*) as recommended by Lykes in 1953 has not yet been used in our febrile cases because a diagnosis of toxoplasmosis has not been made in these cases until a rather late stage of the disease.

In the few cases in which penicillin has been tried, no effect on the fever or lymphadenopathy has been observed.

### *Prophylaxis*

The congenital infection is acquired *in utero*, as manifest symptoms may be present at birth and also since the pathological changes at that time may already be of older date. Despite this and in contrast to the severe infection in the child, the majority of the mothers have apparently been healthy, without any history of definite illness previous to or during pregnancy. On postnatal examination no characteristic signs of disease have as yet been demonstrated. A case of afebrile lymphadenopathy in the third month of pregnancy, however, has been observed in a 29-year-old mother who later gave birth to a child with typical congenital toxoplasmosis (FIGURE 4). The titers of the seroreactions in the mother were slightly increased in the acute stage of the disease, but both the dye test and complement fixation test became strongly positive later.

A similar case has been reported by Farquhar and Turner (1949).

The detection of acquired toxoplasmosis in pregnancy is still the only possible way of preventing the tragic cases of congenital toxoplasmosis in which the severe damage to the brain and eyes is irreparable and for which no specific therapy has as yet been devised. Therefore, the most thorough examination should be carried out when pregnant women show evidence of inexplicable fever, lymphadenopathy, or fatigue. The safest course would be to advise that pregnancy be avoided in the clinically and serologically acute stages of acquired toxoplasmosis, where the possibility might exist of the presence of *Toxoplasma* in the blood.

### *Epidemiology*

Although all reports on toxoplasmosis in animals have not yet been confirmed by the isolation of the organism, there seems to be no doubt that warm-blooded animals are susceptible; at least all attempts to infect them experi-

* Burroughs Wellcome & Co., Inc., Tuckahoe, N. Y.

TABLE 7
ISOLATION OF TOXOPLASMA GONDII FROM LYMPH NODE BIOPSIES FROM 10 HUMAN CASES

| Case no | Sex | Age | *Toxoplasma* Dye test | *Toxoplasma* CF test | Lymph node biopsy | Time between onset and biopsy | Hist path findings | Isol of *Toxopl* |
|---|---|---|---|---|---|---|---|---|
| 1 | F | 17 | 1:6250 | 1:64 | Cervical | ? months | + | + |
| 2 | F | 28 | 1:1250 (6250) | 1:32 (64) | Cervical | ? months | + | + |
| 3 | F | 58 | 1:6250 | 1:32 | Occipital | 2 months | + | + |
| 4 | M | 3 | 1:6250 | | Axillar | 6 weeks | + | + |
| 5 | F | 24 | 1:1250 | 1:32 | Axillar | 6 weeks | + | + |
| 6 | M | 16 | 1:1250 | 1:64 | Retroauricular | 7 weeks | + | + |
| 7 | M | 39 | 1:6250 | 1:128 | Axillar | 51 days | + | + |
| 8 | M | 12 | 1:6250 | 1:128 | Cervical | 7½ weeks | + | + |
| 9 | F | 12 | 1:6250 | 1:128 | 1) Inguinal<br>2) Axillar | > 3 weeks<br>> 3½ months | + | + |
| 10 | F | 20 | 1:250 | 1:64 | Occipital | 5½ months | + | + |

TABLE 8
INOCULATION OF MICE WITH A LYMPH NODE SUSPENSION

| Case no | No of mice inoculated | No of mice: Dead | Survivors | Sacrificed | Observation period in days | Positive dye test | Isolation of *Toxoplasma* |
|---|---|---|---|---|---|---|---|
| 1 | 4 | | 4 | | 57 | 4/4 | + |
| 2 | 10 | | 10 | | 40 | 10/10 | + |
| 3 | 7 | | 7 | | 39 | 7/7 | + |
| 4 | 4 | | | 1(14) | | 0/1 | + |
| | | | 3 | | 38 | 3/3 | |
| 5 | 4 | | | 1(9) | | 0/1 | + |
| | | | 3 | | 39 | 3/3 | |
| 6 | 8 | | 8 | | 57 | 8/8 | + |
| 7 | 4 | 1(13) | | 1(11) | | 1/1 | + |
| | | | 2 | | 52 | 2/2 | |
| 8 | 4 | | 4 | | 51 | 3/4 | + |
| 9 | 5 | | 5 | | 10 mths | 4/5 | + |
| 9a | 3 | | 3 | | 7 mths | 3/3 | + |
| 10 | 4 | | 4 | | 6 mths | 4/4 | + |
| Total | 57 | 1 | 53 | 3 | | 52/56 | |

Died during collection of blood

*Toxoplasma* in the peritoneal exudate. The rest of the animals—56 apparently healthy mice—were examined after a period from 38 days to 10 months following inoculation. On serological examination 52 of the 56 mice tested showed dye test titers from 1:10 to 1:6250. The strains cannot be distinguished either morphologically or by staining techniques from Sabin's strain RH. In cross-neutralization tests using two of the human strains and one hare strain as antigens in the dye test no difference could be demonstrated in the titers of various human and animal sera from proved cases (Christiansen and Siim 1951) (TABLE 9).

and the ingestion of raw meat, milk, and eggs from infected animals and droplet infection Transmission by blood sucking animals should also be taken into consideration In the laboratory, one case was reported after the bite of an experimentally infected rabbit (Eichenwald) Transmission from man to man has not been observed for certain except in the congenital cases

(4) *Resistance of* Toxoplasma Although it is known that *Toxoplasma* are sensitive organisms that do not resist heat desiccation, chemical compounds, *etc*, further experiments are necessary to elucidate how long *Toxoplasma* can survive for instance, in the meat of slaughtered animals and in raw eggs It is possible that the pseudocysts will exhibit a considerably greater resistance than isolated organisms, for example against ventricular juice

*Isolation Experiments*

*Technique* Lymph nodes are removed surgically, minced with scissors and saline is added to make a 1 5 to 1 10 suspension Then 0 5 cc of this rather coarse suspension—and not only the supernatant fluid—is injected intraperitoneally into clean stock mice bred at the State Serum Institute, in which spontaneous infection has never been found to occur In the present inoculation experiments alternate mice were taken as controls, and all the 91 control animals showed negative dye tests when discarded at the same time as the inoculated mice The high percentage of takes, in contrast to the purely negative controls excludes the possibility of a spontaneous infection in the stock mice The infection only rarely kills the animals but most often runs a subclinical course According to Aagaard s technique the animals are killed after about six weeks a dye test is performed on heart blood and subinoculations are made from the brain After inoculation with a lymph node suspension *Toxoplasma* antibodies develop rather slowly the dye test first being positive three to four weeks later Therefore in routine diagnostic tests the animals are observed for four to six weeks Instead of using heart blood the dye test can be performed on samples of tail blood thus allowing the mice to live for further observation It is not necessary to carry out subinoculations as all mice tested have developed antibodies already in the first passage

*Toxoplasma* were demonstrated microscopically in all cases in the peritoneal exudate

Our first strain was recovered in 1952 (see 1952c) and subsequently several strains have been obtained (Siim 1952 1953a 1955) also by Armstrong and MacMurray (1953) Cathie (1954) and Desmonts (1955)

The results of 11 isolation experiments using lymph node material from 10 patients are shown in TABLES 7 and 8 Of these patients 6 were female and 4 male the youngest being a boy of 3 years and the eldest a woman of 58 years The lymph nodes have been excised from the occipital retroauricular axillary and inguinal regions 6 weeks to 5 5 months after onset of the disease In Case No 9 a strain was first isolated from a lymph node from the inguen and 3 5 months later from an axillary biopsy In all cases characteristic histopathological changes were demonstrated

In all 57 mice were inoculated of these, only one died on the 13th day with

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TABLE 9

DYE TEST TITERS USING DIFFERENT STRAINS OF TOXOPLASMA AS ANTIGEN

| Serum | *Toxoplasma* strain | | |
|---|---|---|---|
| | Human | | Hare No 101c (Christiansen) |
| | RH (Sabin) | No 1444 (Case 7) | |
| Human Case No 462 | 6250 | 6250 | 1250 |
| Hare No 3538 | 1250 | 1250 | 1250 |
| Hare No 3539 | 6250 | 6250 | 6250 |
| Dog No 116 | 250 | 250 | 250 |
| Rabbit No 1389 | 6250 | 6250 | >1250 |
| Rabbit No 1391 | 6250 | 6250 | 6250 |
| Silver fox No 1493 | 1250 | 1250 | 1250 |
| Silver fox No 2263 | 1250 | 1250 | >250 |
| Monkey No 475 (inoculated with RH) | 1250 | 1250 | 1250 |

In protection experiments rabbits surviving infection with one of the strains isolated were immune to a challenge three months later with 0 2 cc of a peritoneal exudate (containing 29 million free *Toxoplasma* per cc) injected intracutaneously

Unpublished experiments show that experimentally infected rhesus monkeys have developed a disease very similar to that observed in humans Furthermore toxoplasmic lymphadenopathy has been found in a case of laboratory infection in which a technician pricked his finger on an infected (RH strain) needle (Beverley Skipper and Marshall 1955)

Animal experiments show that *Toxoplasma* infection is carried via the blood stream and Prior *et al* 1953 have isolated *Toxoplasma* from the blood of humans The occurrence of a generalized lymphadenopathy and the isolation of *Toxoplasma* from lymph nodes from various regions in the same patient (Case 9) also indicate that the infection is generalized This concept is supported by isolation experiments in which the parasites have been recovered from muscle biopsies in patients with toxoplasmic lymphadenopathy (Siim unpublished studies)

### *Summary and Conclusion*

(1) *Toxoplasma* have been isolated from enlarged lymph nodes excised from patients with characteristic clinical histological and serological findings

(2) The strain isolated has been identified as *Toxoplasma gondii* by morphological serological and pathogenic criteria

(3) A significant rise in serological titers has been demonstrated in the acute phase of the disease simultaneously with the development of the lymphadenopathy

(4) Toxoplasmosis has been reproduced in rhesus monkeys and a similar disease has been reported in a case of laboratory infection

# THE LABORATORY DIAGNOSIS OF TOXOPLASMOSIS*

*By* Heinz F Eichenwald

*Department of Pediatrics The New York Hospital-Cornell Medical Center New York N Y*

The isolation of *Toxoplasma* from a patient is of course the most convincing demonstration that this organism is present in the host Unfortunately even in active infections it is difficult to isolate these protozoa from an intact host and one is forced to resort to more indirect methods

It is my intention to discuss rather briefly our experiences with the various diagnostic tools available omitting, however, all references to pathologic techniques since these will be discussed elsewhere in this monograph

Basically laboratory methods fall into three classes (1) isolation of the organism (2) serologic tests of various types and (3) cutaneous tests with Toxoplasmins

As already mentioned isolation of *Toxoplasma* from an intact patient is fraught with difficulty With suitable techniques and careful attention to detail however organisms may sometimes be recovered from the blood bone marrow cerebrospinal fluid and if available from biopsy material of lymph nodes or other organs In the chronic phase of illness, parasites are occasionally found in striated muscle tissue Visual demonstration of the organisms is rarely successful in these tissues or body fluids animal chick embryo or tissue culture inoculations must be used to insure any degree of success Of these three host systems animals are probably the most sensitive indicators of small numbers of organisms Tissue cultures are less sensitive and chick embryos are the least useful in this respect

Unfortunately chronic latent toxoplasmosis is not uncommon in most species of laboratory animals For these reasons it is advisable to test the animal colony at frequent intervals for the presence of this type of infection and to perform blind passage of tissues from normal animals at the time that the attempt at isolation is made

In our experience mice hamsters and guinea pigs are most sensitive to the infection Occasional strains show an initial low virulence to one or the other of these hosts It is therefore wise to utilize several different species for each isolation attempt

It has been demonstrated in our laboratory that several modifications in technique result in an increase in the sensitivity of the method

In the first place since *Toxoplasma* do not survive well outside the host inoculation of the test animals should be accomplished in the shortest possible time It is our practice to grind tissue to a fine suspension utilizing a 20 per cent serum 80 per cent buffered saline mixture or to centrifuge body fluids and to resuspend the sediment in serum saline The use of serum in the suspension medium decidedly increases the sensitivity of the procedure To prevent bacterial growth penicillin and streptomycin may be added at concentrations of 100 units and 100 µg per milliliter respectively

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the dye test partially or completely. For our test system we therefore use a suspension of washed *Toxoplasma*. The use of siliconized glassware materially aids in minimizing damage to the organisms during this manipulation which is carried out as expeditiously as possible.

Because of the frequent occurrence of a prozone phenomenon it is our practice to carry out a full range of twofold dilutions. By general usage the dye test titer is defined as the highest original dilution of serum that in the presence of a constant amount of accessory factor is capable of preventing the staining of the cytoplasm of 50 per cent of the extracellular *Toxoplasma*. Since, however, a number of variables occur, we always include a standard serum in each test run as well as three sets of control tubes consisting of (1) the *Toxoplasma* suspension, (2) the *Toxoplasma* suspension plus accessory factor, and (3) the *Toxoplasma* suspension, accessory factor, plus saline.

At least 90 per cent of the organisms in all control tubes should be well stained, otherwise the test should be discarded.

The dye test becomes positive earlier than the complement fixing reaction *and has usually reached high levels by the end of the second week after infection.* In active disease titers in excess of 1:1000 are found in a month or so. These antibodies tend to persist in most individuals for a number of years, probably in excess of a decade, although in gradually decreasing titer. Some children with the congenital infection show a rather rapid decline in their antibodies so that by the age of two to four years the dye test may be positive only in dilutions of 1:4 or less. Whether the persistence of antibodies in most individuals is associated with a chronic infection is not known. Experience with animals indicates that the antibody level may fall while organisms still persist in the host. We had the opportunity to study one infant with the congenital disease who succumbed at the age of 19 months as the result of an unrelated event. Shortly after birth this infant had shown dye test titers of above 1:1000 and at the age of 6 months his antibody level had begun to decline, reaching 1:128 a few weeks before his death. The complement fixation test similarly diminished from 1:64 to 1:4. Nevertheless at autopsy *Toxoplasma* were readily isolated from brain tissue but not from other organs.

If the antibody titer of an adult patient without active disease is followed closely, it may occasionally be noted that over a period of weeks or months the titer fluctuates up or down by several dilutions without any apparent cause. This fact must be considered in the interpretation of serologic data, a task further complicated by the fact that a large percentage of some normal adult population groups show antibodies to *Toxoplasma*. Transplacental transfer of these antibodies to normal infants occurs;[8] a positive dye test in a newborn does not necessarily mean disease. The examination should be repeated after two months when passively acquired antibody will have decidedly diminished.

In my discussion so far I have repeatedly referred to the complement fixation test. I should now like to discuss it in more detail.

While this test has the great advantage of not requiring the laboratory to maintain a strain of *Toxoplasma*, it has proved useful only as an adjunct to the dye test.

Complement fixing antibodies appear in the course of the disease later than

The administration of cortisone to the test animals for three to five days prior to inoculation increases the chances of recovering *Toxoplasma*, particularly strains of low virulence For example the LD50 titer of one strain of *Toxoplasma* maintained in tissue culture measured $10^{-6}$ in control animals as against $10^{-8}$ in mice pretreated with cortisone

Following the initial intracerebral and intraperitoneal inoculation we sacrifice approximately half the animals on the fifth day, and subinoculate brain and spleen suspensions as well as peritoneal washings The interval of five days is chosen because immune bodies appear after this time, and the number of successful isolations begins to diminish Our practice consists of performing at least six blind passages and observing all surviving animals for one month before sacrificing them to examine their tissues We formerly performed serologic *and challenge tests on all survivors but our experience has not indicated that* this will increase the number of positive diagnoses if cortisone treated animals are used

If an organism morphologically similar to *Toxoplasma* is isolated it is not sufficient to regard it as *Toxoplasma* on cytologic grounds alone The parasite can be identified with certainty only by the use of serologic methods

At present three general types of serologic tests are available (1) the dye test originated by Sabin and Feldman [1] (2) the complement fixation test [2,3] and (3) neutralization tests now carried out chiefly in tissue cultures

For diagnostic purposes the dye and complement fixation (CF) tests are most useful Tissue culture neutralization is of interest chiefly as a research tool to study cell parasite relationships

Let us first discuss the dye test This is undoubtedly the most useful tool in the diagnosis of all stages of *Toxoplasma* infection The test is based on the observation that both the cytoplasm and nucleus of the organism when incubated with normal serum subsequently stains deeply with alkaline methylene blue whereas after exposure to antibody containing sera only the nuclear endosome takes the dye Certain morphologic differences may also be noted between neutralized and nonneutralized *Toxoplasma* [4] These differences are best revealed by phase contrast microscopy

A fairly large amount of a heat labile complementlike accessory factor is necessary for the reaction to take place While the *Toxoplasma* antibody is *quite stable the accessory factor is not Sera containing it must be stored in* the frozen state

The technique *of the test has been described in sufficient detail in a number* of publications [1,5] so that I shall not discuss it I should like however to mention a few factors that may affect the results

Dilutions of the test serum should always be made up in saline so that the amount of accessory factor remains at the same volume in all tubes The methylene blue buffer mixture must be prepared freshly for each run If mouse peritoneal exudate is used as the source of the *Toxoplasma* this material should be obtained from the animal no later than three days after infection since otherwise antibodies in the mouse may adversely affect test results [6]

Like Leon Jacobs [7] we have found that a soluble antigen occasionally appears in the peritoneal exudate or tissue culture fluid in sufficiently high titer to block

satisfactory Because of the inherent simplicity of technique we generally prefer to use cells grown on glass Malignant epithelial cells of the HeLa type are stable easy to handle and can readily be grown in almost unlimited quantity Trypsinized kidney tissue is also easily prepared and grown, unlike malignant tissue the kidney cells can readily be maintained in fluid devoid of serum [11] This property is of advantage because an occasional specimen of commercially prepared bovine, equine, or human serum shows some degree of anti *Toxoplasma* activity even after inactivation

For the production of antigens or for other procedures where quantities of organisms are desired stationary *flat sided bottles* are most useful since they permit the growth of large numbers of cells Tube cultures are entirely adequate when only small volumes are needed

The cells may be grown in any of a variety of nutrient media depending on the type used After an interval of from 5 to 14 days varying with the cell type and whether bottles or tubes are used the cellular population is usually adequate to permit inoculation with *Toxoplasma* If any serum has been present in the nutrient medium we generally wash the cells twice with a maintenance fluid then add the *Toxoplasma* containing inoculum and, finally the maintenance solution

Organisms usually appear in the supernatant in small numbers on the third day after inoculation Maximum levels are reached three to five days later At that time approximately 10 to 12 million organisms per cubic centimeter may be present in the fluid Almost all of them are free floating extracellular forms The number of organisms produced varies directly with the number of available cells Meanwhile the tissue under cultivation undergoes diffuse cytopathic changes and eventually is completely destroyed

Tissue cultures do not appear to be as sensitive a medium for the isolation of *Toxoplasma* as are cortisone pretreated animals Nevertheless these *in vitro* methods are valuable laboratory tools since they permit the production of large quantities of *Toxoplasma* that are easily counted sedimented and washed Relatively pure antigen can thus be prepared in concentrated form Tissue cultures also will become increasingly useful in the study of immune reactions of cell parasite relationships cell metabolism and possibly in the screening of potential chemotherapeutic agents against *Toxoplasma*

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those detected by the dye test They are rarely found earlier than one month after infection They also diminish quite rapidly with time For example, 44 out of 60 children with the congenital disease whom we have followed since birth showed no detectable complement fixing titers at five years of age By the age of 7 52 had lost these antibodies, although all except 3 still had dye test titers Interestingly enough 32 of the 60 mothers had detectable CF antibodies 7 years after giving birth to the infected infant

Thus a positive complement fixation test in titers of 1 32 or above is indicative of a relatively recent infection, but a negative reaction does not rule out past contact with *Toxoplasma* As A B Sabin[3] has pointed out, infants born with highly active congenital infections occasionally have negative CF tests with strongly positive dye tests despite the presence of both high dye and CF test values in the mother In infants children and adults it is not possible to correlate any level of dye test antibodies with the presence or absence of CF titers

*Complement fixing* antigen may be prepared in any of several different ways We have employed chick embryos mouse brain peritoneal exudate and more recently tissue cultures in the preparation of this material Peritoneal exudate while a rich source has a strong anticomplementary activity that cannot be eliminated by high speed centrifugation[3] We prefer to use chorioallantoic *membrane or* tissue cultures as sources since these antigens are rarely anticomplementary and control material is readily prepared

In the past antigen was usually prepared by subjecting *Toxoplasma* containing fluid to repeated freezing and thawing but since we have shown that this process leads to a diminution of potency we now prepare our material by grinding the sediment with glass powder

While not strictly a laboratory procedure the cutaneous tests using Toxoplasmin as described by J K Frenkel[9] are useful aids in population surveys and as screening tests for the diagnosis of the so called third stage of toxoplasmosis Patients with highly active disease do not usually possess dermal hypersensitivity Infants with congenital infection only rarely give positive reactions before the age of nine months A negative skin test therefore does not imply absence of antibodies to *Toxoplasma* A positive reaction on the other hand is probably nearly always associated with some antibody

Proper control material must always be used in the *performance of th s test* and for this reason we like to use material derived from chick embryos or tissue cultures rather than mouse peritoneal exudate since an adequate control for the latter is difficult to prepare A not inconsequential number of individuals show dermal hypersensitivity to the antibiotics routinely incorporated in tissue culture media it is advisable to omit these drugs from the culture from which antigen is to be obtained

As a final point I should like to discuss tissue culture technique in greater detail *Toxoplasma* can readily be propagated in roller tube cultures[10] a well as in systems utilizing suspended cells or cells grown directly on glass surfaces Roller tubes while technically more difficult to use produce large numbers of *Toxoplasma* per cubic centimeter of fluid but the other methods are entirely

days after intraperitoneal infection. Although serum antibody titer can often be found in mice three days after infection, insufficient amounts diffuse into the peritoneal fluid to be of practical importance in performing the dye test. The use of *Toxoplasma* grown in tissue cultures as described by Heinz F. Eichenwald should avoid the presence of antibody.

Certain other modifications help to make dye test results more clear cut. Heparin produces a precipitate that obscures unstained *Toxoplasma*. Hence we take up the peritoneal fluid in about one tenth the amount of 4 per cent disodium ethylenediamine tetracetate dihydrate (sequestrene $Na_2$, versene) which is a very effective anticoagulant.

Frequently fibrin clots containing enmeshed cells with *Toxoplasma* are already formed in the peritoneum of the mouse. Placing a cover glass on a drop of a mixture containing clots results in liberating many intracellular *Toxoplasma* from within the clot. Since these organisms remained intracellular during incubation they were not exposed to antibody and retained their staining ability. Hence a large number of unneutralized organisms are added to a neutralized mixture, making a test more difficult to read. Drawing up and ejecting the harvested peritoneal exudate through a No. 27 hypodermic needle attached to a syringe will free a majority of these cells. This procedure also enhances disintegration of host cells containing *Toxoplasma* and thus, while making the latter available for the test, reduces the number of disturbing stained organisms in a neutralized admixutre.

Finally, the need for testing twofold serum dilutions starting with undiluted serum must be stressed. Using 1:64 as the lowest dilution tested will result in a high instance of false negative tests. As the significance of low titers is being better appreciated it becomes clear that serologic surveys are misleading unless low titers are considered. The measured dye test titer is but a point on a biologic curve, and it has been found that after known infection the titers can decline gradually to zero. Unless it can be shown that a cross reacting organism such as *Sarcocystis*, *Trichomonas* or *Trypanosoma*, as reported by Awad[3], produces false positive titers up to a certain magnitude, it is undesirable to limit one's concept as to the meaning of antibody by neglecting the low titers. By the same token serum dilutions up to at least 1:1024 should be tested routinely since prozones have been observed involving this range. Twofold dilutions serve to show up technical errors and draw attention to variation in results that are easily overlooked or rationalized if fourfold or even greater dilutions are sampled. A standard neutralizing serum with a titer near the center of the test range should always be included in a test.

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PAUL GRONROOS (*Department of Serology and Bacteriology, The University of Helsinki, Helsinki, Finland*). In an attempt to discover the mechanism involved

## *Discussion of the Paper*

J. K. FRENKEL (*Department of Pathology and Oncology, University of Kansas School of Medicine, Kansas City, Kans.*): The dye test is a very useful but complex test, the performance of which often presents difficulties to the novice as well as to the experienced workers. Two factors interfering in the dye test can be clearly distinguished. One is the presence of soluble antigen, first reported by Leon Jacobs[1] which may completely obscure a low antibody titer and lead to irregular results with poor definition of the end point. TABLE 1 shows how a titer of 1:32 can be affected by soluble toxoplasma antigen derived either from a mouse or a chick embryo source.

The second difficulty I experienced several years ago, just after coming to Kansas. I wanted to screen some sera for the absence of antibody in order to select one for accessory factor. To my amazement, 18 consecutive sera of young medical students gave positive results. I happened to have only the CJ strain of *Toxoplasma* mice at that time and was using fourth day peritoneal fluid, as I used to do previously with the RH strain. One day I utilized third day peritoneal exudate and neutralization no longer occurred. Systematic investigation showed that the presence of antibody in mouse peritoneal fluid can give rise to false positive tests. More frequently, it leads to constant partial neutralization of *Toxoplasma* described by Beverley and Beattie. Although unstained *Toxoplasma* may be present in native peritoneal fluid, neutralization appears only after accessory factor is added. TABLE 2 compares the serum and peritoneal fluid antibody titers in two strains of mice four and five

TABLE 1
EFFECT OF SOLUBLE ANTIGEN ON DYE TEST TITER

| D lu [illegible] | Titer |
|---|---|
| Saline | 1:32 |
| Saline with toxoplasmic peritoneal fluid sediment, extracted by freezing and thawing | 1:4 |
| Saline with 20% infected chorioallantoic membrane | 1:2 |
| Saline with 10% infected chorioallantoic membrane | 1:8 |
| Saline with 5% infected chorioallantoic membrane | 1:16 |
| Saline with $2\frac{1}{2}$% infected chorioallantoic membrane | 1:16 |
| Saline with $1\frac{1}{4}$% infected chorioallantoic membrane | 1:32 |

TABLE 2
ANTIBODY TITERS IN SERUM AND PERITONEAL FLUID OF MICE AGAINST RH 3RD DAY FROM CFW MICE

| Strain [illegible] infected [illegible] RH intrape[illegible] | D y [illegible] | Dy[illegible] | |
|---|---|---|---|
| | | [illegible] | P rt fl d [illegible] |
| Hauschka | 4th day | 1:48 | 1:8 |
| | 5th day | 1:64 | 1:6 |
| CFW | 4th day | 1:64 | 1:6 |
| | 5th day | 1:48 | 1:12 |

# PATHOGENESIS OF TOXOPLASMOSIS AND OF INFECTIONS WITH ORGANISMS RESEMBLING *TOXOPLASMA**

*By* J. K. Frenkel

*Department of Pathology and Oncology, University of Kansas School of Medicine, Kansas City, Kans.*

When reflecting on the disease manifestations that may accompany *Toxoplasma* infection of animals and man, it is well to consider that the majority of infections pass unnoticed, since they are asymptomatic. Only rarely has a mother recalled symptoms of infection, although she may have given birth to an infant with toxoplasmosis apparently transmitted *in utero*. It has been shown experimentally that rats, dogs, monkeys, and chickens can undergo asymptomatic infections,[20] and *Toxoplasma* has repeatedly been isolated from seemingly healthy animals in nature.[41]

What is the nature of interactions between *Toxoplasma* and a variety of hosts that favor either asymptomatic infection or the development of clinical disease? This question should be asked for each stage of the infection, since different mechanisms operate. During the acute infection, proliferative forms of *Toxoplasma* multiply actively within a variety of cells and organs, and lesions result from the destruction of parasitized cells and the accompanying inflammatory reaction. Asymptomatic chronic infection is generally maintained by *Toxoplasma* cysts in the central nervous system, the eye, and the myocardium, with lesions resulting after the disintegration of these cysts, usually without the subsequent liberation of a multiplication of organisms.

*Proliferative organisms* (FIGURES 1, 2, 4, 10) and *cyst organisms* (FIGURES 5 to 9) have been differentiated in Leon Jacobs' presentation;[30] their cytologic features and the terminology used by others have been discussed previously.[24] A certain amount of confusion as to what cysts are has arisen due to the finding of intracellular proliferative forms in the peritoneal fluid of mice with acute toxoplasmosis, which were misinterpreted as pseudocysts.[10] The same author then correctly observed that the terms cyst or pseudocyst were not descriptive and introduced the designation "terminal colony" to indicate the final stage of leukocytic parasitization by *Toxoplasma*.[10] These organisms proliferate actively within leukocytes and elsewhere during acute infection; they can be shown to contain little glycogen, and no argyrophilic PAS-positive cyst wall is present. Hence data derived from these leukocytic inhabitants cannot be transferred to organisms that multiply slowly or not at all, that contain large glycogen granules and are contained within *cysts* that persist extracellularly for prolonged periods of time.

A distinction as to whether proliferative forms or cysts are producing lesions is also important, since only proliferative *Toxoplasma* are inhibited by chemotherapeutic agents available at present. Such agents include the sulfonamides, the sulfones, pyrimethamine, Aureomycin, and erythromycin.

It is the purpose of this paper to draw attention to the dynamics of patho

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in Sabin Feldman's dye test it was found that properdin is an essential factor in the dye test reaction itself It was demonstrated that the accessory factor active in the reaction consists of properdin + $C_2$ + $C_3$ + $C_4$ Pillemer *et al* showed that the properdin system is a factor in natural immunity and that it has for example bactericidal action An attempt to prove that the properdin system alone gave a positive dye test failed Bovine properdin, in an original concentration of 75 u /ml , gave no positive dye test in the absence of immune serum However, in the presence of immune serum, the toxoplasmolysis was unusually strong and the dye test positive A properdin concentration of 2 4 u /ml gave a maximally positive dye test with known immune serum, 1 2 u /ml gave a lower titer and 0 6 u /ml gave a completely negative result

The properdin was made available by Louis Pillemer Before use it was stored for a month at −20 C , according to Pillemer the properdin content in this bovine properdin preparation may have dropped somewhat For this reason it is possible that the above concentrations are only approximate values

The possibility cannot be excluded that the properdin system together with nonspecific antibodies may give a positive dye test

A modified dye test can be used for properdin titration, in this event an immune serum with a dye test titer exceeding 1/1024 should be used

TABLE 2

DEVELOPMENT OF IMMUNITY TO TOXOPLASMA IN MICE VACCINATION WITH BDA STRAIN CHALLENGE WITH THE VIRULENT CJ STRAIN

| Gro p | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | 8 | 1 | 3a | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V inatio 0-day w th | BDA | N e | BDA | N | BDA | N ne | BDA | | BDA | BDA | BDA | BDA |
| CJ m in jected | I t b lly | | I trape t lly | | Subc t ly | | N e | | N | IC | IP | SC |
| Day f CJ jection | D y f d th ft CJ jectio | | | | | | Res r oc l t | | V e d th 40 m | D y f death ft r nat xpected day f d th | | |
| | | | | | | | dy test | f test | | | | |
| 1 | 4 | 3 | 6 | 5 | 18 | - | 0 | 0 | | 5/4 | /6 | 19/8 |
| 2 | 3 | 2 | 5 | 3 | 8 | 7 | | | | 5/4 | /5 | 10 ) |
| 3 | 4 | 4 | 4 | 4 | 14 | 6 | 0 | 0 | | 7/ | 8/7 | 1/9 |
| 4 | 3 | 3 | 5 | 5 | 10 | 7 | | | | 7/ | 9/9 | 14/11 |
| 5 | 4 | 3 | 8 | 6 | | | 0 | 0 | | 9/8 | 13/11 | /12 |
| 6 | 3 | 3 | | 6 | | , | | | | 9,9 | /12 | /13 |
| 7 | 3 | 2 | 5 | 5 | | 6 | 2 | 0 | | 10/9 | 12/12 | /13 |
| 8 | 24 | 3 | 16 | 4 | 11 | 6 | | | | 32/11 | 24/12 | 18/14 |
| 9 | / | 3 | | 6 | | 6 | 16 | 0 | | 16/12 | /15 | 16/15 |
| 10 | | 3 | 26 | 6 | | | | | | /13 | 36/16 | /17 |
| 11 | | 3 | | 6 | | 7 | 256 | 0 | | /14 | /17 | /18 |
| 12 | | 3 | | 5 | | 6 | | | | /15 | /1 | /18 |
| 13 | 5 | 3 | | 6 | | | 8000 | 8 | | 18/16 | /19 | /20 |
| 14 | | 4 | | 5 | | 7 | | | | /18 | /19 | /21 |
| 15 | | 3 | | 5 | | 6 | 1024 | 8 | | /18 | / 0 | /21 |
| 16 | | 4 | | 6 | | 7 | | | | /18 | /22 | /23 |
| 17 | | 3 | | 5 | | 6 | 4096 | 8 | + | /20 | /22 | /23 |
| 18 | | 3 | | 5 | | 7 | | | | | | |
| 19 | | 4 | | 5 | | 7 | 4096 | 24 | | | | |
| 0 | | 2 | | 5 | | 7 | | | | | | |
| 21 | | 3 | | 5 | | 8 | 048 | 16 | + | | | |
| 22 | | 3 | | 5 | | 7 | | | | | | |
| 23 | | 3 | | | | 7 | 2048 | 40 | | | | |
| 24 | | 3 | | 5 | | 7 | | | | | | |
| 25 | | 3 | | 9 | | / | 8000 | 12 | + | | | |
| 26 | | 4 | | 6 | | 8 | | | + | | | |
| 27 | | 3 | | 6 | | 6 | 4096 | 20 | | | | |
| 28 | | 4 | | 6 | | 7 | | | | | | |
| 29 | | 3 | | 6 | | 7 | 2048 | 3[illegible] | | | | |
| 30 | | 3 | | 5 | | 6 | | | | | | |

N t blank pac indic t l f r 60+ days Rec proc l l f se l g t t rs ted

/)[51] Since the challenge inoculum consisting of fourth-day peritoneal fluid of infected mice varied from day to day three unvaccinated mice were injected daily as controls employing intracerebral intraperitoneal and subcutaneous injections (groups 2 4 6) Deaths resulting from vaccination amounted to approximately 10 per cent (group 8) Simple survival times are noted for groups 1 to 6 Columns 1a 3a and 5a give the survival periods counted from the day of vaccination those of vaccinated mice are represented by the numerator and those of the controls by the denominator From a comparison of

genesis and to discuss how the latter is affected by developing immunity by the formation and persistence of *Toxoplasma* cysts and by anti inflammatory adrenal corticoids

*Acute infection* The wide host range and the ability to multiply in many cells are unique characteristics of *Toxoplasma* Since proliferative organisms can undergo binary fission two or three times daily they soon fill the cytoplasm of host cells resulting in cell rupture with liberation of organisms and soluble antigen [10 16 30] An inflammatory reaction develops composed largely of mono nuclear cells Organisms enter other cells in the local area in the regional lymph nodes or in the lungs and other organs after hematogenous dissemina tion A pregnant host may transmit infection transplacentally to the fetus [8 24 40] probably leading to abortion during the first trimester and to disease in the infant if infection is transmitted during the latter stages of pregnancy in woman Parasitemia may be transient or prolonged [30]

The acute infection may be entirely asymptomatic When the prolifera tive rate is rapid however, a sufficient number of individual cells may be de stroyed by the *Toxoplasma* they harbor to produce foci of tissue necrosis at the inoculation site, in the regional lymph nodes the liver and the spleen accom panied by the exudative reaction of pneumonia (FIGURES 3 11 12 14 24) This results in functional damage that produces symptoms and may bring about death of the host In man the nonspecific signs and symptoms of fever ma laise weakness and headache may accompany a rash and other specific signs of tissue or organ involvement

The survival time in mice is correlated in TABLE 1 with the infecting dose of a virulent strain of *Toxoplasma* The $LD_{50}$ was calculated according to the formula of Reed and Muench [47] the survivors were proved noninfected by sub inoculation

*Emergence of immunity* The conditions under which lesions may develop are markedly altered as immunity develops TABLE 2 illustrates the increasing resistance of mice to challenge with the CJ strain of *Toxoplasma* following prior vaccination Two hundred and twenty male mice were injected sub cutaneously with the low virulent BDA strain serving as vaccine Every day thereafter three mice were challenged with the virulent CJ strain (groups 1 3 5) and 3 mice were bled and their pooled sera were tested for antibody (group

TABLE 1

CORRELATION OF DAY OF DEATH WITH DOSE OF CJ TOXOPLASMA INJECTED INTRAPERITONEALLY

| Dilution of inoculum | Day of death—6 mice | | | | | | Mean day of death | Multiples of $LD_{50}$ |
|---|---|---|---|---|---|---|---|---|
| $10^{-1}$ | 5 | 5 | 5 | 5 | 5 | 6 | 5 1 | 100 000 |
| $10^{-2}$ | 5 | 6 | 6 | 6 | 6 | 6 | 5 8 | 10 000 |
| $10^{-3}$ | 6 | 6 | 7 | 7 | 7 | 7 | 6 6 | 1 000 |
| $10^{-4}$ | 7 | 7 | 8 | 8 | 8 | 8 | 7 6 | 100 |
| $10^{-5}$ | 8 | 9 | 9 | 9 | 9 | 10 | 9 0 | 10 |
| $10^{-6}$ | 9 | 9 | 10 | — | — | — | 9 3 | 1 |
| $10^{-7}$ | — | — | — | — | — | — | | |

Th[illegible] ocula co[illegible] t d [illegible] pool [illegible] trape[illegible] 0 2 ml [illegible]

TABLE 4

DEVELOPMENT OF HOMOLOGOUS IMMUNITY IN MICE INFECTED WITH THE CJ STRAIN OF TOXOPLASMA AND TREATED WITH A MIXTURE OF SODIUM SULFADIAZINE AND SULFAMERAZINE 15 mg PER CENT IN THE DRINKING WATER (see D )

| Development of immunity | | | | Development of antibodies | | |
|---|---|---|---|---|---|---|
| Duration of treatment after infection (days) | Survival time after reinfection (days) | | | Day of test during continuous treatment | Reciprocal dye test titer | |
| | Route of reinfection | | | | Route of infection | |
| | IC | IP | SC | | IP | C |
| 0 | 3 0 | 5 5 | 8 0 | 0 | 0 | 0 |
| 1 | 3 5 | 5 0 | 9 0 | 1 | 0 | 0 |
| 2 | 4 0 | 6 0 | 8 0 | 2 | 0 | 0 |
| 3 | 3 5 | 6 0 | S | 3 | 4 | 0 |
| 4 | 4 0 | S | S | 4 | 256 | 4 |
| 5 | All mice died by | 5 0 | S | 5 | 512 | 64 |
| 6 | the 4th day | S | S | 6 | 256 | 256 |
| 8 | | S | S | 8 | 2 048 | 1 024 |
| 10 | | S | S | 10 | 2 048 | 512 |
| 12 | | S | S | 12 | 16 000 | 512 |
| 14 | | S | S | 14 | 2 048 | 8 000 |
| 16 | | S | S | 16 | 8 000 | 16 000 |
| 18 | | S | S | 18 | 4 096 | 8 000 |
| 20 | | S | S | 20 | 8 000 | 16 000 |

NOTE S = survival IC = intracerebral IP = intraperitoneal SC = subcutaneous inoculation.

delayed death and by the scattering of survivors may in part be due to remaining infection in some mice whereas others were cured from their initial BDA infections Complete cross immunity was acquired to CJ strain infection In these instances immunity was measured 6 and 12 weeks after two vaccinations with live BDA strain organisms *Immunization with CJ or BDA followed by CJ* confers greater immunity against the RH strain [16]

*Development of homologous immunity* Immunity to the homologous strain may be acquired more quickly than to a heterologous strain as shown in TABLE 4 Immunity in this instance was measured by the period of suppressive treatment necessary before survival was assured Groups of mice were injected on the same day with 50 000 $LD_{50}$ either intracerebrally intraperitoneally or subcutaneously and were placed on suppressive sulfonamide treatment after six hours Daily thereafter the mice were taken off treatment Their survival periods and serum antibody titers are recorded in TABLE 4

All intracerebrally inoculated mice died as if untreated and it was found in subsequent experiments that treatment with 400 mg per cent of sulfadiazine in drinking water for 10 days is necessary to prevent death of all mice Intraperitoneally inoculated mice survived after 4 to 6 days of treatment and were immune on the 7th day Subcutaneously inoculated animals survived after only three days of treatment and they were immune on the 10th day The acquisition of immunity occurred more rapidly and apparently there was no significant period of partial immunity as had been observed during the development of cross immunity By the time immunity appeared to be solid dye test

TABLE 3

CROSS IMMUNITY IN MURINE TOXOPLASMOSIS PRIMARY INFECTION WITH THE BDA STRAIN CHALLENGE WITH THE RH AND CJ STRAINS

| Intraperitoneal injection with | No. of animals | Survival time in days | |
|---|---|---|---|
| | | BDA immune | Not immunized (6 mice per group) |
| RH $10^{-2}$ | 5 | 6 6 7 S S | 4 4 4 5 6 6 |
| $10^{-3}$ | 4 | 8 8 14 S | 5 6 6 7 7 7 |
| $10^{-4}$ | 3 | 10 10 23 | 7 7 7 8 8 8 |
| $10^{-5}$ | 3 | 12 12 18 | 7 8 8 8 9 10 |
| $10^{-6}$ | 3 | 17 S S | 9 9 S S S S |
| $10^{-7}$ | 3 | S S S | S S S S S S |
| CJ $10^{-1}$ | 2 | S S | 4 4 4 4 5 5 |
| $10^{-2}$ | 2 | S S | 4 5 5 5 5 6 |
| $10^{-3}$ | 2 | S S | 6 6 6 6 7 7 |

the survival time of mice in group 4 with the titration data in TABLE 1 one can estimate the approximate number of organisms injected daily into groups 1 to 6

The challenge inoculum consisted of 1000 to 100 000 $ID_{50}$ judging from TABLE 1 In spite of this heavy challenge immunity became apparent between 8 and 12 days and was well established 17 to 20 days after vaccination The period of partial immunity varied in animals challenged by different routes It was similar in duration in the artificial somewhat tissue culturelike peritoneal and cranial cavity infections The earlier appearance of immunity ' after subcutaneous inoculation is possibly due in part to an interference phenomenon Since vaccine and challenge organisms disseminate through the body by similar routes many susceptible cells may already contain BDA organisms by the time they are reached by CJ organisms

Comparison with the serologic data reveals that dye test titers (stimulated by the BDA strain but measured for technical reasons with the RH strain) appear simultaneously with the emerging immunity Although no antibody titers were determined in mice that died in this experiment it is highly probable that those that died after the 12th day had high dye test titers Relative to other strains tested antibody to BDA appears relatively late (seventh day) whereas with CJ and RH strains the dye test antibody could be demonstrated as early as the third and fourth day after infection (TABLE 4) Complement fixing antibodies appeared after the 11th day in BDA infected mice

Acquired immunity as measured by longer survival is dependent on many factors of which antibody is only one [16] Intracellular *Toxoplasma* appear to be unaffected by antibody [31] It has been previously discussed that mice infected with the RH strain although developing similar titers of antibody as those infected with CJ and BDA do not develop a similar degree of immunity to their infection [16] The experiment outlined in TABLE 2 serves as an illustration of the development of cross immunity between strains against which the host here the mouse can develop solid immunity TABLE 3 illustrates the development of only partial cross immunity to the RH strain The marked individual variability as indicated by the distribution of instances of rapid or

therapy may develop rapidly progressing signs of central nervous system irritation such as circling, choreiform movements, paralysis and coma.[9,16] This may be accompanied by chorioretinitis.[16,20] *Toxoplasma* multiply in neurons and other cells leading to cellular and interstitial necrosis. This results in an early microglial and a later astrocytic reaction and slight perivascular cell proliferation (FIGURE 14). Sometimes extensive lesions develop due to infarction necrosis. A number of human cases have also been described where meningoencephalitis was most prominent clinically and at autopsy.[50,55]

Human neonatal toxoplasmosis is commonly manifests itself by central nervous system lesions. Here again *Toxoplasma* organisms continue to proliferate actively in the brain, leading to the production of microglial nodules (FIGURE 20), whereas in the extraneural viscera lesions are subsiding and antibody, presumably of maternal origin, can be demonstrated. In addition a unique lesion consisting of periventricular vasculitis and necrosis is generally observed whenever aqueductal obstruction and internal hydrocephalus are present (FIGURES 17 to 19). This lesion in the human brain has been interpreted as resulting from the interaction of intravascular antibody and intraventricular toxoplasmic antigen as the latter diffuses into the brain stroma, producing the lesions wherever the ependymal lining is absent.[14,4] Periventricular necrosis is found around the lateral and third ventricles and surrounding the aqueduct proximal to its stenosis but not distally in the fourth ventricle, which is usually not dilated (FIGURE 20). Hence the rostral ventricular system resembles an abscess cavity since the antigen cannot escape via the aqueduct and the foramina of the fourth ventricle. *Toxoplasma* also multiply in the ventricles and their walls, and it is thought that an antigenic pool develops in this fashion. The diffuse vasculitis and necrosis occurs in the absence of organisms. Infants with periventricular necrosis may have a serum dye test titer of 1:1000 or higher. Antigen has been demonstrated in the ventricular fluid of such patients by injection into hypersensitive guinea pigs;[4] it might also be measured in complement fixing units. This distinctive lesion is more completely described and discussed on pages 8 to 11 and 56 to 61 of the earlier publication.[4] It is probably pathognomonic for toxoplasmosis when found in young infants.

It is of interest to note that infants infected *in utero* who survive will later develop antibody of their own (see cases VI and VII and p. 49, Frenkel and Friedlander[4]). This is contrary to the indifference or tolerance of some hosts to antigen contacted *in utero* as discussed by Edsall.[10a]

Prolonged microbial activity in the central nervous system is not, of course, unique for *Toxoplasma*, but it appears to be the pathogenetic mechanism giving rise to most viral encephalitides and to paralytic poliomyelitis. This is presumably due to the less effective cellular inflammatory response, the greater vulnerability of nerve cells, and the diffusion gradient for hematogenous antibody. In a dog with chronic inactive toxoplasmosis, the antibody content of the cerebrospinal fluid was only one thousandth, and that of the brain one four hundredth of the serum titer of 1:32 000.[22] In the presence of active encephalitis, a lesser discrepancy in titers would be expected; however, no figures have been published to my knowledge.[11]

*Survival from active infection with chronicity or cure.* As in many infections

titers of 1:512 and 1:1024 had been reached. The antibody response appeared more rapidly during treated CJ infection, where it was 1:1024 on the 8th day as compared to only about 1:8 on the 8th day of untreated BDA infection. This may in part be due to greater antigenicity of CJ and in part due to the finding that CJ infected mice passed through the period of immunization in better physical condition. The antibody response to CJ quicker after intraperitoneal than after subcutaneous inoculation, may be analogous to the observations by the Taliaferros.[56] They showed that participation of the spleen in antibody production as after intravenous injection of antigen is more effective in bringing about high antibody titers than the participation of lymph nodes primarily as after splenectomy or as in this instance, after subcutaneous inoculation.

*Hypersensitivity.* In man, rhesus monkeys, and guinea pigs, a delayed type of hypersensitivity may be observed three to four weeks after infection.[13] This can be a factor complicating pathogenesis especially in relation to cyst rupture.[14, 15, 16, 0] The usefulness of the toxoplasmin skin test in man depends on the high correlation between presumed infection as indicated by antibody measured in the dye test, and the presence of hypersensitivity.[19, 24] *In most rodents* hypersensitivity is not demonstrable by skin test; nonetheless it appears to *participate in the histogenesis of lesions. Just as in tuberculosis or after suc*cessful BCG vaccination where dermal hypersensitivity may fail to appear in *about 10 per cent of individuals so toxoplasmin sensitivity is not always de*veloped following infection especially in young children. The presence of *antibody measured in the dye test is a more inclusive index of past infection.*

*Development of lesions during period of partial immunity.* In mice and hamsters on partially suppressive chemotherapy most of the visceral lesions are found to be subsiding toward the end of the second week of infection. Toxoplasmic proliferation is found longest in the lungs and the central nervous system. Pulmonary macrophages and alveolar cells are parasitized, accompanied by copious exudate and sometimes by small foci of necrosis and pleurisy. Cases in man where death appeared to occur in this stage have been described by Corpening, Stembridge and Rigdon[7] and by Pinkerton and Henderson.[44]

Benign lymphadenopathy with marked reticular cell and some lymphoid hyperplasia and sometimes with abnormal lymphocytes in the blood has been observed in some children[53] and adults[1, 3, 53] with histories and serologic changes indicating recently active or acquired infection. *Toxoplasma* organisms have been isolated in several instances.[1, 3, 53] This hyperplastic reaction appears to accompany the successful acquisition of immunity. Depletion is generally found during *the early acute infection since release of lymphocytes appears to* exceed the rate of production. Hyperplasia may persist together with organisms *for 3* months or longer but both may have disappeared after about 10 months.[1]

Central nervous system lesions are of particular clinical significance since toxoplasmic immunity is acquired but slowly in the brain and eye. This lag especially apparent in certain combinations of hosts and strains of *Toxoplasma* as in hamsters infected with the RH strain. Animals that are partially immune either from prior infection with another strain or after inadequate chemo

sulfonamides) Whereas infected survivors generally show antibody titers cured survivors might or might not develop antibodies probably depending on the duration of active infection and on the interval between infection and testing

In order to simulate in this experiment (CHART 1) the latitude of results that might be encountered in an epidemiological investigation of natural untreated infections doses of 10 1000 and 100 000 LD₅₀ were administered to groups of 6 to 12 mice and the findings were charted according to strain and chemotherapy only When charted according to size of inoculum it became apparent that with the larger inocula more animals died whereas with smaller ones more animals survived and freed themselves of infection CHART 1 shows also that the comparative drug efficacy for a 15-day treatment period is greater for the mixture of sulfadiazine and sulfamerazine than for Aureomycin which was given at close to the maximum tolerated dose It is also indicated that CJ infection is suppressed more effectively with either drug than is RH infection This can be expressed in terms of the relative rate of immunogenesis in mice becoming effective faster in CJ than in RH infection as discussed elsewhere [14] Small experiments with guinea pigs and rabbits indicated that a higher percentage of cures is obtained than in mice and hamsters using identical strains

Judging from the high incidence of antibodies and toxoplasmin sensitivity in certain human and animal populations coupled with the virtual absence of clinically recognized disease it is possible to suspect that many of those implicated may no longer be actually infected It is not possible at present to distinguish by serologic or skin tests however whether individuals with antibody or skin sensitivity remain infected or are cured Brain and other tissues would have to be subinoculated No observations appear to have been published concerning immunity as measured by challenge of animals cured from infection some of which have and others have not developed antibody (see Uninfected survivors in CHART 1) It should be cautioned here against acceptance of all serologic titers as indicating past or persisting toxoplasmic infection since cross reactions have been reported [4]

*Chronic toxoplasmosis the* Toxoplasma *cyst and lesions resulting from cyst rupture* Although some proliferative forms of *Toxoplasma* may persist the development of the cyst stage is the important factor in maintaining chronic infection *Toxoplasma* cysts when intact do not give rise to an inflammatory reaction (FIGURE 13) This is apparently due to a tough resilient argyrophilic and periodic acid Schiff positive cyst wall that apparently prevents escape of antigenic and chemotactic substances (FIGURES 5 6 8) It appears reasonable to regard the cyst wall as of parasite origin although this has not been proved Cysts are found most frequently in the brain occasionally in the retina the myocardium in skeletal muscle and possibly elsewhere as in lymph nodes and the adrenal gland A certain host and organ predilection may be present since cysts have been seen in the brain and eye of mice and hamsters but never in the myocardium where they are not uncommon in guinea pigs Certain *Toxoplasma* strains may give rise to more cysts than others with the RH strain in mice and hamsters this may be due to the more prolonged rate of proliferation before immunity becomes effective

INCIDENCE OF EARLY MORTALITY AND SURVIVAL WITH CHRONICITY OR CURE

□ - Uninfected ▨ - Chronicity ■ - Mortality

(Numbers refer to dye test titers of individual mice)

PERCENT
100
80
60
40
20

RH (Sulfonamides): Uninfected: 4, 16, 32, 32, 32, 256, 2000; Chronicity: (Serum lost), 130 000, 500 000

CJ (Sulfonamides): Uninfected: 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 4, 4, 4, 4, 4, 4, 8, 8, 8, 16, 16, 8000; Chronicity: 1000, 8000, 8000, 32 000, 65 000, 130,000

CJ (Aureomycin): Uninfected: 32,000; Chronicity: 2000, 16 000, 16 000, 16 000, 16 000, 32 000, 32 000, 32 000, 32 000, 65 000, 65 000, 130 000, 130,000

RH (Aureomycin): Uninfected: 0

Strain RH | CJ | CJ | RH

SULFONAMIDES | AUREOMYCIN

surviving animals remain chronically infected although a fairly effective degree of immunity can be shown to exist. This can easily be demonstrated in toxoplasmosis of mice after a brief period of partially suppressive chemotherapy. CHART 1 compares the incidence of early mortality and of survival with either chronicity or cure as determined by subinoculation 60 days after infection. Reciprocal dye test titers of individual survivors are noted. These murine infections with two strains of *Toxoplasma* treated either with sulfonamides (60 mg per cent in drinking water) or Aureomycin (200 mg per cent in food) reveal patterns that may be observed in other hosts whether treated or not. The mortality rate might be high and survivors, if any are present, might not remain infected (see RH infection treated with Aureomycin). The total percentage infected might be similar to that noted before. More animals however might manage to survive (CJ + Aureomycin). Mortality might be low, infected survivors few, and cured survivors might be in the majority (CJ +

chronic toxoplasmic eye lesions. It is quite possible that chronic persistent ocular involvement is due to proliferative forms and that transient episodes followed by recovery are due to the sudden release of antigen following cyst rupture The recognition of this dual mechanism is of special importance in the appraisal of chorioretinitis in man as has been discussed in connection with another paper[49] So far proliferative organisms and cysts have been described in chronic infections only from the *retina* whereas primary toxoplasmic iridocyclitis or choroiditis as implied by the clinical term uveitis has been encountered neither in the human eye[49] nor in the experimental hamster and mouse In both man and hamster the uvea is involved secondarily by 'neighborhood inflammation' which may be much more intense than the retinal inflammatory response This refers to naturally evolving ocular lesions, in animals essentially immune following systemic infection if *Toxoplasma* are injected directly into the anterior chamber an acute primary iridocyclitis results.[33]

*Chronic toxoplasmic myocarditis and myositis* The best example in a patient of heavy muscle involvement by cysts leading to clinical myositis and to the diagnosis being made by skeletal muscle biopsy was described by Kass Andrus Adams Turner and Feldman[33] Less severe myocarditis has been observed clinically possibly due to proliferative forms and cysts[54] Lesions associated with what appear to be *Toxoplasma* cysts have been described from a kangaroo[6] Cysts in skeletal and cardiac muscle are elongate corresponding to the shape but not necessarily the length of the host cell (FIGURES 7 13) Their cyst wall is argyrophilic and PAS positive and the cyst organisms show prominent PAS positive glycogen granules Organisms found in heart muscle should be differentiated as to whether they are cyst or proliferative forms since myocarditis can also be produced by the latter during the acute infection (FIGURES 11 12)[52]

*Cysts in other organs* What appear to be cysts have been described from the adrenal gland of a kangaroo that had myocarditis[6] A cyst resembling *Toxoplasma* has been found in a lymph node associated with serologic evidence of past infection[54] Some experimental evidence has been obtained that cysts may persist in the lungs of infected mice[18] Certainly cysts are found infrequently outside the brain eye myocardium and skeletal muscle and their rupture is less likely to be followed by symptoms of clinical significance

*Resumption of proliferative activity during chronicity in the adrenal gland and after the administration of anti inflammatory steroids* Hamsters with chronic toxoplasmosis of several months duration succumb occasionally showing large numbers of *Toxoplasma* proliferating in the adrenal glands (FIGURES 21 22) These glands are often virtually destroyed in spite of some apparent attempts at regeneration Other lesions due to multiplying organisms are generally absent Antibody titers usually remain high

Resumption of toxoplasmic proliferation has been observed also after the prolonged administration of cortisone acetate Hamsters infected for several months that have resisted challenge injections may succumb with severe toxoplasmic pneumonia and encephalitis accompanied by necrosis but with deficient inflammatory response (FIGURES 10 23) In these animals too anti

Cysts can maintain chronic infection for two years and probably longer[48] In rats for example their presence is generally not accompanied by symptoms Structures that appear to be *Toxoplasma* cysts have also been found, incidentally at autopsy of human beings[35, 37, 45] In other hosts symptomatic chronic toxoplasmosis has been observed associated with large numbers of cysts but without proliferating organisms This observation has led to the hypothesis that cyst rupture is actually responsible for these lesions[14, 15, 16, 0] It has been difficult to find remnants of cyst wall or of organisms in such lesions This is readily understood when considering that the cyst wall is less than one micron in thickness and that organisms injected into immune hosts quickly disappear, even from the brain A few lesions however have been identified in the brain of hamsters[16] and in the skeletal muscle and brain of a human patient[33] where remnants of organisms were found in the center of early necrotizing lesions The role of cyst rupture as a pathogenetic mechanism is easily demonstrated in infections with another cyst forming organism *Besnoitia jellisoni* The larger *Besnoitia* cysts contain more organisms and the cyst wall is thicker, so that degenerating organisms as well as cyst wall remnants, can be found in the center of lesions (FIGURE 40)[17, 18, 0] *Toxoplasma* organisms liberated by cyst rupture are usually destroyed in the immune host since no evidence of renewed proliferative activity can ordinarily be found

*Chronic* Toxoplasma *encephalitis* In the lesions of *acute* toxoplasmic encephalitis organisms are seen within many cells and where cell death has occurred also extracellularly In *chronic* toxoplasmosis lesions are generally found devoid of organisms and represent a reaction to cyst rupture Young lesions in chronic infections consist of a focus of necrosis, suggesting a hyperergic response sometimes with recognizable cyst remnants (FIGURE 15) Hematogenous and microglial cells wander in and phagocytize the debris forming a microglial nodule which represents the tombstone of a *Toxoplasma* cyst (FIGURE 15) Hypertrophic astrocytes are found in the periphery Symptoms depend on the number of such lesions which in turn depend on the number of cysts and rate of cyst decay on the location of lesions and on the number that are superimposed in a given fiber tract With spinal cord involvement, paralysis is generally ascending in hamsters When many lesions are present, blood vessels may become involved leading to thrombosis and to infarction necrosis of nervous tissue In man isolated cysts have more often been reported than large numbers of cysts with the presence of lesions[35, 37, 45] The same holds for dogs but in chinchillas and hamsters encephalitis has been reported after treatment of acute infection with sulfonamides and numerous cysts and lesions have been found in the central nervous system[16, 34]

*Chronic toxoplasmosis of the eye* Lesions related to cyst rupture have been described from hamsters[20] and cysts have been observed in the eyes of mice[-3] and of human infants and adults[3, 69] Lesions related to the parasitism by proliferative *Toxoplasma* of individual retinal cells have been observed in hamsters[0] and also in the majority of human eyes which had been enucleated because they were blind from long continued inflammation and painful due to secondary glaucoma[69] There accordingly appears to be a dual etiology to

rarely in laboratory mice and rabbits organisms are encountered in the brain and kidneys where they are best demonstrated by staining with carbol fuchsin[12 42] *Toxoplasma* is observed so rarely in the kidneys of any host that organisms reported in this location should be carefully differentiated from *Encephalitozoon* Both organisms used to be encountered commonly in seemingly healthy laboratory rabbits and mice Perhaps due to changed breeding practices such infections are rare now *Encephalitozoon* however has recently been isolated in mice (from Japan) that received materials suspected to contain the rickettsiae of scrub typhus *Encephalitozoon* has also been found in mice that were injected with various organs from North American bats and probably in mice treated with urethane during investigations of human and mouse hepatitis[41] The organism has also been isolated from laboratory rats in the United States and in England[5 30] During the course of research on lymphogranuloma venereum virus (LGV) at the Institute Pasteur in Paris it was found that mice that proved chronically infected with *Encephalitozoon* were more resistant to intracerebral inoculation of LGV than mice not so infected[38]

*M-organism* (FIGURES 29 to 31) This is the temporary designation for an organism observed in field mice (*Microtus modestus*) trapped near Hamilton Mont which resembles *Toxoplasma* morphologically[15 4 57] The distinguishing feature is its cyst which is lobulated and septate in the brain whereas that of *Toxoplasma* is spherical and aseptate This organism did not give rise to infection when subinoculated into other *Microtus* white mice hamsters or guinea pigs Findley and Middleton observed an organism with similar characteristics in *Microtus agrestis* from England[12] They believed this to be *Toxoplasma* and tried to implicate it as causing a fatal epidemic in field mice No significant encephalitis was present however and transmission experiments with organisms from lobulated cysts appeared inconclusive

*Sarcocystis* (FIGURES 32 *to* 39) Sarcocystis has occasionally been confused with *Toxoplasma* Descriptions of species have been based on their occurrence in a number of hosts mainly herbivorous mammals and water birds Carnivores are rarely found infected Only the cyst stages from cardiac and skeletal muscle are well known Focal myocarditis and myositis sometimes widespread are produced by the breakdown of cysts The cyst walls are argyrophilic but PAS negative Some species such as those from cottontail rabbits show radial spines (FIGURE 34) Sporoblastic cells are often found inside the cyst wall The cysts of some species contain compartments and the central compartments may be free of organisms Large nuclei are sometimes found in the compartment walls The individual cyst organisms are large and have 2 rounded ends whereas those of *Toxoplasma* the *M organism* and *Besnoitia* are smaller and have one pointed and one rounded end *Sarcocystis* organisms are *motile* have vesicular nuclei and contain glycogen granules of a characteristic perinuclear distribution (FIGURE 37)[18] Unlike fungi individual organisms are not surrounded by a PAS positive wall They react with cytoplasm modifying antibody in a fashion similar to *Toxoplasma* (FIGURES 38 and 39) The occurrence of schizogony is doubtful Morphologic studies of organisms from man sheep mouse rabbit squirrels and duck did not reveal fungal characteristics The few accounts of transmission by subinoculation or feeding of

bodies may remain high although frequently they are reduced Occasionally, overwhelming bacterial infections kill such animals before toxoplasmosis relapses [3]

These observations concerning apparent immunity depression in chronic toxoplasmosis of animals during the administration of pharmacologic doses of cortisone parallel those made in a variety of bacterial infections such as human tuberculosis and those in monkeys with chronic trypanosomiasis [61] Indeed, a patient with Hodgkin s disease has recently been observed who while on pharmacologic doses of cortisone succumbed to toxoplasmic encephalitis with lesions suggesting relapse rather than first infection

Evidence has recently been presented, indicating that the resumption of proliferative activity by *Toxoplasma* in the adrenal gland is facilitated by and probably dependent on the corticoid hormones elaborated there [1]

The presence of ocular lesions due to proliferating *Toxoplasma* during chronicity is not associated with a measurable decrease in immunity elsewhere except sometimes in the adrenal gland Nonetheless serum antibody in patients presumed or proved to have such lesions is usually low [19, 4, 31] It is reasonable to surmise although further proof is required that acquired immunity can decrease during chronic infection to such a degree that *Toxoplasma* can resume proliferation This may follow cyst rupture due to changed cellular factors maintaining immunity as in the adrenal glands or after the administration of anti inflammatory corticoids due to decreased serum antibody levels, which are even lower beyond the blood brain and eye barrier and due to a variety of unknown factors

Activation of latent infection in pregnant mothers has been postulated as a mechanism leading to fetal infections [57] All available clinical evidence however indicates that this occurs rarely if at all [4, 60] It has not been observed in man and only under exceptional circumstances in experimentally infected animals [8] Transplacental transmission in man has been linked only to acute infections

*Infections with organisms resembling* Toxoplasma Whereas a comprehensive consideration of the pathogenesis of such other infections would have to be lengthy especially in view of our inadequate knowledge of them the infectious organisms may briefly be considered primarily to differentiate them from *Toxoplasma*

*Encephalitozoon* (FIGURES 25 to 28) This organism was originally suspected to give rise to neonatal disease in man Wolf and Cowen however in their second paper recognized *Toxoplasma* as the etiologic agent [60] Some authors consider this organism as a species of *Toxoplasma* [5] It might be better however to consider these two organisms separately until proved identical Much evidence has been presented to show that they are distinct They look different They do not cross immunize and accumulations of *Encephalitozoon* in the central nervous system are not surrounded by a cyst wall [18, 4, 43] Acute infections of mice characteristically lead within two or three weeks to the development of ascites in which small numbers of organisms are found Infections are generally nonfatal except when mice are treated with cortisone which facilitates extensive proliferation of the organisms in most organs [18] Ordi

*Summary*

More asymptomatic than clinical cases of toxoplasmosis occur Hence the development of lesions and disease represents an atypical reaction pattern when compared to the more common types of host *Toxoplasma* relationships The interactions between host and microbe starting with the generalized acute infection, are traced through partial states of immunity with lesions centered in the central nervous system to the chronic infection which may or may not be symptomatic Reactivation of lesions may follow due to the waning of immunity in the retina and the adrenal cortex or following the exogenous ad ministration of anti inflammatory corticoids The dual pathogenesis of lesions is described as relating to the destruction by proliferative organisms of individ ual infected cells and to that following cyst rupture in the partially immune and often hypersensitive host

The following small microorganisms are briefly discussed since due to their morphologic likeness to *Toxoplasma* they present differential diagnostic prob lems *Encephalito oon Mucrotus* (M)-organism *Sarcocystis Besnoitia Globidium* leishmaniform stages of *Trypanosoma Cryptococcus* (*Torula*) *Histoplasma*, and *Klossiella*

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cyst organisms are poorly substantiated Such attempted transmission of mouse and rabbit *Sarcocystis* to laboratory mice and rabbits were unsuccessful in the author s hands [18] *Sarcocystis* from cottontail rabbits and house mice also failed to grow on media ordinarily supporting fungal growth It would seem from these studies that these organisms neither look nor behave like fungi as has been suggested by Spindler [63]

*Besnoitia* (FIGURES 40 to 45) Organisms resembling *Toxoplasma* but enclosed in a large cyst (up to 2 mm) surrounded by a heavy cyst wall and with cyst wall nuclei are found in deer mice from Idaho (*Besnoitia jellisoni*) (FIGURES 40 to 43) [17, 18] and in cows and horses in southern Europe and South Africa (*B besnoiti*) (FIGURES 44 45) [9] Similar organisms have been described in chickens with Bangkok disease [6] and in reindeer from Alaska (*Fibrocystis tarandi*) [7] Although the cyst stages do not resemble those of *Toxoplasma* the proliferative organisms from the acute infection and the lesions produced are almost indistinguishable *Besnoitia jellisoni* has been studied in detail since it more regularly produces ocular and adrenal involvement than *Toxoplasma* and because the sequence of events following cyst rupture can be studied more easily with its bigger cysts which are formed throughout the body in large numbers [17, 18, 0] *Besnoitia besnoiti* gives rise to a generalized infection in cows and horses followed by the production of cysts, which is accompanied by dermatitis and thickening of the skin called olifantvel" in Afrikaans [9] This infection is also known as globidiosis and the organism has been designated by the generic terms *Gastrocystis Globidium* and *Sarcocystis* *Globidium* (*Gastrocystis*) however refers properly to an intestinal coccidian parasite of horses and sheep which multiplies by schizogony (FIGURES 46 47) [39] *Besnoitia* has been applied to an organism multiplying by binary fission which produces a generalized infection *Besnoitia jellisoni* and *Toxoplasma* are serologically and immunologically distinct Sera from cows naturally infected with *B besnoiti* obtained through the courtesy of W O Neitz of the Onderstepoort Veterinary Institute Onderstepoort Union of South Africa did not contain measurable antibody using *B jellisoni* as antigen in the dye test [18] *B jellisoni* is pathogenic to chick embryos and to white mice hamsters rats newborn guinea pigs and certain other rodents but it did not produce demonstrable infection in a cow two rhesus monkeys adult guinea pigs rabbits chicks canaries and pigeons *B besnoiti* has been transmitted experimentally to rabbits [46]

*Leishmania* and leishmaniform stages of *Trypanosoma cru i* can be recognized by their kinetoplast Fungi such as *Cryptococcus* (*Torula*) and *Histoplasma* have been confused with proliferative forms of *Toxoplasma* that appear rounded after fixation Both fungi have PAS positive cell membranes but are devoid of PAS positive granules and cysts are ab ent *Klossiella* a sporozoan parasite usually reported from the kidneys of mice and guinea pigs [28] can be differentiated from *Toxoplasma* *Besnoitia* and the M organism by the presence of schizogony and the production of an oocyst *Toxoplasma* like organisms from the bile ducts of a dog [58] are probably merozoites of a *Coccidium* with schizogonic stages likewise present

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PLATE 1

## PLATES 1 to 10

(FIGURES 1 to 47)

### PLATE 1

PROLIF ATI E FORMS OF TOX PLASMA

FIGURE 1 *T xopla ma* f esh preparatio fr m pe t n l exu l te of mo se, ho ing org n ms f e d w th ma oph ge. Red blood rpuscles som f which a e nated pr de a mpa ison f ize. Ph se-contra t m rosc pe ×1000

FIGURE 2 *T pl ma* n imp s mear from l ng f m e G msa stain ×1000

FIGURE 3 A t epatitis in h m t ght day aft ubc taneo nfect n th the Rh st in of *T o-pla ma* H matoxyl and eo in. ×13

FIGURE 4 *T xopl ma* in t ular c ll of pleen f h mst n days fter infect w th the Rh strain of to plasma P r d aci l Schiff hemat yl ×1000.

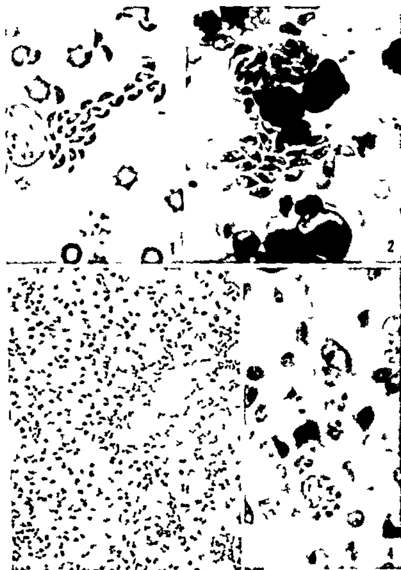



PLATE 1

PLATE 2

COMPAR SON F TOX L SMA CYST A D PR LIFERAT F RM

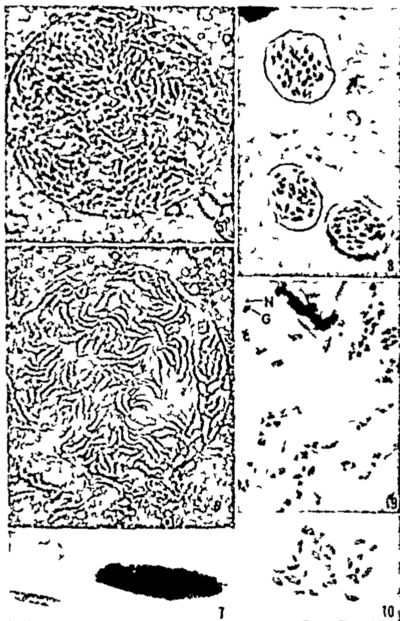
FIGU 5 d 6 *T pl m* y t f h p p t n f m th b f mo e yea ft fect w th th Rh t a Th sam y t how wh le d p tially ted ft p s e o th r gl ss au d t rupt re Th teg ty f th ll psed yst walls app t ga m e mo e cl ly ee th n the t t cy t ×1000

FI U 7 Cy t i m oc rdum f a k g p mably to p l sm C mp th FIGU E 13 *T p m* cy ts th b t s ten ly w th th m th d S e F GU 9 fo th pp c of the d d l g m Pe d d S hiff h mat yl ×4 5 S t o t y of D ld B H k l

F GURE 8 *T plas* y t Rh t app imat ly y ft f ti S l mp egnat by W ld m th d d m t ate th g d t f the y t w ll d t d by th agg d t p d ed by t o g w th th m ot me ×1000

FI U E 9 *T pl m* y t g m f om th b a f a m se y ft fect Sm l y t rt fic ll rupt ed t d w th th p d d Schiff t h q e t d t th larg gly g (G) g l th be d g t d by d t e Hem t yli t the leus (N) l ghtly ×1000

FIGURE 10 *T pl m* p l ferat g m f m the pe t l fl d f m f d y ft fec t Glycog g les wh p t r m ll d d ff e d t b t FIGURE 4 h th t th y a b ly ppa t t P d c a d S hiff h m t xyl ×1000




P 1B 3

PLATE 3

FIGURES 11 d 12 Acut t pl m myoca dit f man A som what diffuse lesion is a ompa d by I } f ta e fo m f T xopla m g ism ( w} Sect o t y f D nald E Eyles[18]

FIGURE 13 Ch myoc dit s pr mably to plam l m ka g oo A rcumscr bed l sulting aft the d t g t n f a y t n t accomp n ed by ga m Tw y t ( rr ) ar sh th t are not acc mpan d by cell lar r act C mpare th FIGU E 7 Secti n rt y f D nald B H ckel

FIGURES 11 to 13 P od c c d S hiff h m toxyl n X135 X475 X135



11
12
13

PLATE 4

TOX PLASMIC ENCE H ITIS IN HAMSTE S

F GURE 14 L f ll w g 12 day f inf t n w th m der te numbe f p l f t g *T pl m* nd nfl mmatory re t Hemat y l n az r II o ×475

FIGU E 15 Le o s follow g o e ye r of i f ct o Focal e r sis ol the g la d F ki j lay f the c b ll m (b k t ) A old r les d at d by th ar ow Th se les ns f ll w cy t rup- t P d d S h ff h m t xyli ×135

FIGU E 16 L 13 day f ll w g th bc t dm t t n f cortis e a t t Th h m t h d b e i f t d 50 day p ly (f ll w d by lf m d th py) d wa challe ged w th *T pl m* 17 days late foll wed by 5 mg f t so t t d ly f m th 37th day t ll d th P ol ferat g *T pl m* o g isms are mo e n me ous th i p es tat l n f m th t inf t FI UR 14 Infl mm to y ct app ars dec eased T pla m c p e m ia wh h w ls p t shown i FIGU E 23 H ma toxylin nd eo in ×475










PLATE 4

PLATE 5

NEO ATAL T XOPL SMOSIS OF MAN

SIX WEEKS-OLD I FAN APPA E TLY INFECTED *Ute o* (CASE IV[24])

FIGURES 17 d 18 C ss sect o f b n at th lam l el Ne ro s is r t d l te l d th d e tr l se pt wh h d r l pendyma p se t S l ble a t g dem trable t th se d l ted t les FIGURE 17 W lde s l er imp gnat X1 FIGURE 18 Phosph t gst acid hemat yl X4 5

FIGU E 19 C ss sect th gh po s Th q d t is l ned by zo f It cont ins fib s rud te th t b tructs th q d ct c dal to th p l e f sect o Ph ph t gst id hem t xyl X2

FIGURE 0 Fourth t icle w th gl l od l l g the e t l w ll Gl l; l f rati n exte d int th e tr l l men whe th p d ym h b bl ter t d. Th t le t d l ted and a p r c t l r zo f ec o s i b t N l st X43

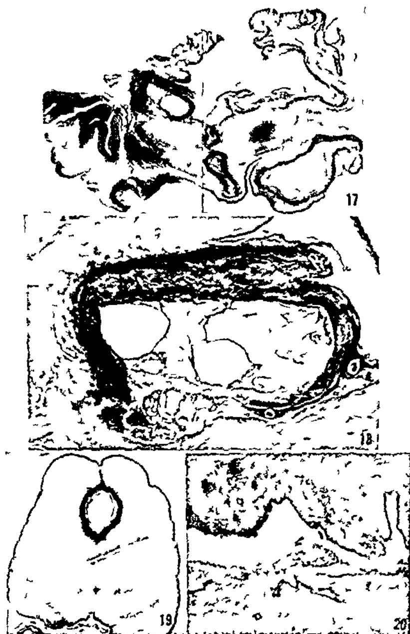



PLATE 5

**PLATE 6**

FIGURES 1 d 22 Necro is d to p olif rat n of *T pl m (* s) i th ad nal corte Th h m t r w f cted 4 m th p u ly d had b t t d th lfad f th f r t 14 d ys aft fect L o d to p l f t *T pl m* w o ly p t th d e l gla ds *T pla* y ts e f d in th bra P od id S huff hemat yl ×13 ×475

FIGURE 23 To pla m c p m ia w th nec sis ( w) r t lly mm ham te th t d ed 15 day foll g the dm t t of t e a t t 5 mg d ly C mpa e ith FIGURE 24 f r th r e f te p umo ia Th br l on f th s anim l e h in FIGURE 16 P od d Schiff h m t yl n ×135

FIGURE 24 A t t yl m c y m a f mm h m t th t d ed 12 days ft r s bc t inoc lat *Tl l g h f e T pl m l* fl d d t f r la g m cr th g s f e troph l g *lo* yte but m mall m o l ells th the imm iz d mal s bseq ently t eat d th tiso h wn in FIGURE 3 P od d Schiff h mat xylin ×135




P 6

PLATE

FIGURE 25. *F* [illegible] ma [illegible] fluid of m [illegible] G [illegible] ×1000

FIGURE 26. *F* [illegible] larly [illegible] Wright [illegible] ×1000

FIGURE 27. *F* [illegible] walls [illegible] nat [illegible] to W[illegible] method. The [illegible] ×1000

FIGURE 28. *E* [illegible] eudocy[illegible] A [illegible] sect[illegible] with the pe[illegible] Sch[illegible] technique. ×1000

FIGURE 29. [illegible] glass [illegible] H[illegible] M[illegible] Not [illegible] ×30

FIGURE 30. [illegible] and [illegible] may be [illegible] comp[illegible] H[illegible] ×30

FIGURE 31. [illegible] Imp[illegible] G[illegible] ×1000




Plate 7

## PLATE 8

### SARCOC STIS SP CIES

FIGURES 32 d 34 to 39 f m c tt ta l r bb t Syl l g s ll ll c ght ar Ham lt M t

FIG RE 32 Cy ts k l tal m l N t g ti ly ta g sef ta f m g mpartme t th e t l o e b g d d f g ms S e FIGU s 34 and 35 f d t l of cysts H m t yl ur II ×30

FI U E 33 Cysts n para ertebral skel tal mus le (M) f l e mouse (M s m l ) c ght r B m M t Sept d r dial p s ar b t Are f a t d h o c nflamm t n, t l ft f c t res lt d f m d unt g t g y t F = b w f t SC = p l d H m t yl II ×30

FIGURE 34 I t f cy t h g d l p and s j ta im gnat d w tl s l er a d g t W ld meth d I t t g ms in ter mp tm ts bo e degenerated n s c t l comp tm t bott m ×1000

FIGU 35 Port n f cyst ta ed th tl e p d c ac d Sch ff t chn que f ll ed by h m toxylin The d l p e ta n o ly sl ghtly and the s t d t t t ll ×1000

FIGURE 36 Imp o mear ta d th tl e p d c c d S h ff te hn que f ll ed by G m a t Th l (N) g t b t g PAS p ti e (gly g ) g les are p l r p t (I gu 3) Cyt pl m u l se n blue t g yt pl m the l pp t t th cleu ×1000

F GU E 37 Imp m r t d w th tl p d d Sch ff t hniq e d m t ti g d t b t n f d a t se d b t bl gly g g l s N = cl s ×1000

F GU 38 F h p p t f S cy t s su pe d d l e and n m l m stai d w th lkal m thyl e bl e Th yt pla m t d ply the l (N) r ma s u ta d ×1000

F U 39 F h p p t f S y t l t rum (fr m th h t bb t) a d h m a sorp f to ta d w th lk l m thyl bl Th l k f h m eo s cyt pl m c ta g I FI RE 38 d ates th a t f yt pl m m d fy g t b dy o d g to the dye t t p c ple ×1000

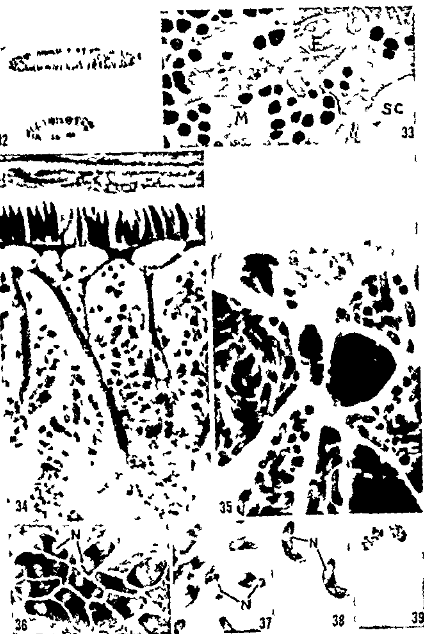



PLATE 6

## PLATE 9

B s OITIA JELLISONI

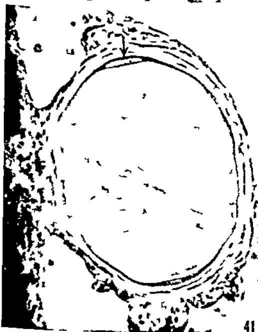
FIGURE 40 Cy t in the p rm t d of m , ght w k ft r tr p rt l oc l t on Th ow p t t m ts of cy t ll f m d t g t d yst whi h ga e ri e to an i t t g l P d c d Schiff h m t xyl ×135

FIGURE 41 Cy t a g f m th s rf f th pl f a m e ght eks after fe t The y t fll d th g n m y t w ll leu d t d by the arrow Hematoxyl n a ur II e ×260

FI U 42 E ly y t mpo d f h ly sta ng w ll de e cytopl m w th ma y l e n l (t o of wh h a bl t ght), d l c nta g f w y t ga ms The p g m lt pl c t o of cy t g m by b ty f the le to la g t l t tak p e t lly th t e cy t d p h th la g ular l g t the cyst w ll a h FIGURE 41 H m to yl n a II ×1000

FIGURE 43 Cy t w ll d r c t c y t g n m s r mbl g *T pla* a H matoxyl a d eo ×1000







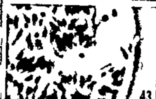


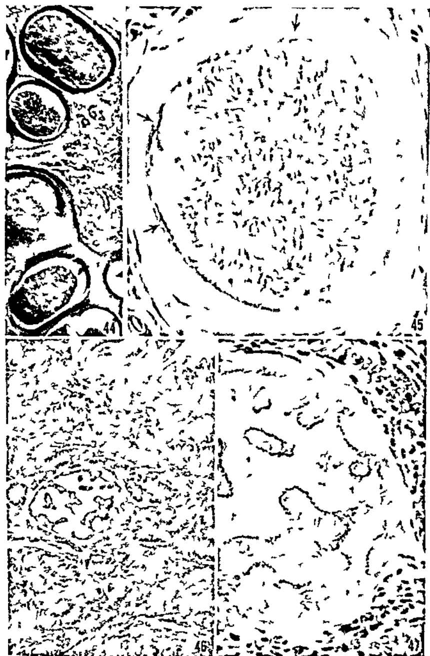

PLATE 9

PLATE 10

FIGURE 44 *B* *i* *b* *ts* *y* ts f m th sk f a f m So th Af The pe od c ac d Schiff po t cy t lls a e hea tha n *B* *j* *ll* d they f qu tly l we Co t r t ed th h mat xylin ×90

FIGURE 45 *B* *i* *b* *no* *t* *y* *t* *t* *d* *th* *h* *mat* *y* *l* d eo n Th y t w ll *j* *se* dic ted by s Th c esc nt cy t org ms sembl th of *B* *j* *ll* d *T* *pl* ×500

FIGURES 46 nd 47 *Gl b d* *g* *i* *th* f m th t t nal m o f a he p N m o g u l e l ed up th s fa f a po gel k y t g l m c mesh g g the app e ce of s h g y M sso g t h m st in ×90 ×500


44
45
46
47

# NEWER KNOWLEDGE OF THE CHEMOTHERAPY OF TOXOPLASMOSIS

*By* Don E Eyles

*Laboratory of Tropical Diseases National Institute of Allergy and Infectious Diseases National Institutes of Health at the Medical School of the University of Tennessee Memphis Tenn*

Until the last few years, the chemotherapy of toxoplasmosis in man was practiced only rarely and with results difficult to assess This was due in part to a lack of drugs of demonstrated value and in part to the difficulty of diagnosis Recent work has resulted in constantly improving methods of diagnosis and the wider use of these methods Current investigations are elucidating the role of toxoplasmosis as a cause of granulomatous uveitis and chorioretinitis[1, 2, 3] and as a cause of a syndrome characterized by lymphadenopathy and fever[4]

In step with these advances in our knowledge of the disease animal experimentation has led to the discovery of agents effective against toxoplasmosis in animals that give promise of being effective in man Results of their use in man are just now being reported For this reason the present is an appropriate time for us to review progress in the chemotherapy of toxoplasmosis

*Action of the sulfonamides* Soon after it was demonstrated by Wolf and his colleagues[5, 6] that *Toxoplasma* are a cause of human disease, efforts were made to find drugs effective against them As early as 1941 sulfonamides were shown by Sabin and Warren[7, 8] and independently by Biocca and Pasqualin,[9] to have definite antitoxoplasmic activity in experimental mouse infections even though there was no *in vitro* action[10] Similarly sulfonamides were shown to be active against toxoplasmosis in rabbits[8] pigeons[11] embryonated hens eggs[12] and perhaps other animals This activity of the sulfonamides was confirmed by a number of other investigators[13-21] and a comparison of their observations up to 1952 was made by Eyles[22]

Only Cross[3] failed to find activity in the sulfonamides Varying degrees of activity however have been described by different investigators the effect observed varying from cure of the infection to mere prolongation of the life of infected animals A study of the research reports indicates that the discrepancies are due perhaps in part to strain variation but also to variations in the technique of testing Notably the size of the inoculum has varied greatly from investigator to investigator as has the mode and timing of drug administration This factor of inoculum size has great influence upon the results of chemotherapy in mice A recent study by our laboratory[24] showed that with sulfadiazine cure could be easily obtained when the inoculum was of the order of 100 $LD_{50}$ whereas when the inoculum was 10 000 or more $LD_{50}$ cure could be obtained only irregularly and never in a large proportion of the mice treated

Of course the fact that the cure rate is dependent upon the size of inoculum and other factors makes it difficult to predict from animal work the effect the sulfonamides will have in man Even so we can learn which sulfonamides are most active, for one would not expect changes in host to alter the order of activity

During the past few years we have used in our laboratory most if not all of the sulfonamides ordinarily used in man for the treatment of other infections Experiments to determine median effective dosages (MLD) have been made in mice under identical experimental conditions so as to allow comparison TABLE 1 summarizes the results of these experiments Other investigators[16] who have used sulfonamides under comparable conditions have found an essentially similar order of activity

Although a recent paper from this laboratory [6] stated that sulfapyrazine was the most active sulfonamide yet encountered more recent work has indicated that sulfamethazine may be slightly more active At any rate these two compounds along with sulfamerazine and sulfadiazine are of a similar order of activity and the confidence limits (CL) of the median effective dosages overlap This would indicate that any positive assertion of their relative order of activity might easily be in error

All of the other sulfonamides tested were much inferior to the above four Sulfathiazole was the most active, but toxic dosages had to be approached to obtain maximum activity Sulfapyridine was not highly active and is known to produce toxicity at levels near those used in our experiments Sulfadimetine known under the trade name of Elkosin, and sulfisoxazole marketed under the trade name of Gantrisin were greatly inferior to the other sulfonamides except sulfapyridine Even though these compounds exhibit very low toxicity we do not believe that this could conceivably compensate for the low degree of activity against *Toxoplasma* Further data on these two latter compounds were presented in a recent paper from this laboratory [6]

It would appear logical to use multiple sulfonamides for the treatment of toxoplasmosis as additive effect with independent toxicity would be expected Two experiments conducted in our laboratory have confirmed that additive action does take place and other investigators have affirmed that such action occurs [6]

The sulfonamides would appear to have the same mode of action against *Toxoplasma* as against other organisms Summers[14] has shown that *p* aminobenzoic acid completely antagonizes the effect of sulfathiazole He showed also that folic acid had a similar antagonistic effect thus indicating that the sulfonamides were competing with the metabolite *p* aminobenzoic acid and thereby interfering with cellular metabolic processes leading to the synthesis of folic acid

In many respects the mouse is not a favorable host for studying the effect of antitoxoplasmic drugs as the infection nearly always causes death and the mouse develops little or no effective immunity to most strains Some animal with an immune re ponse similar to that of man might be a better test animal As stated above Sabin and Warren[8] found that sulfonamides were active against rabbit toxoplasmosis They administered 1 5 to 3 gm of sulfathiazole per day by intraperitoneal injection to rabbits that had been infected intracerebrally A number of these survived indefinitely and were resistant to reinfection Biocca[7] also obtained indefinite survival in five rabbits treated with sulfathiazole

In our laboratory we have obtained the apparent cure of rabbit toxoplasmosis on a number of occasions by administering sodium sulfadiazine to rabbits

TABLE 1

RELATIVE ACTIVITY OF THE SULFONAMIDES AGAINST EXPERIMENTAL TOXOPLASMOSIS IN THE MOUSE

Intraperitoneal in ula of about 100 LD units were followed immediately by 14 days of treatment. Drugs were administered as diet suspensions offered ad libitum

| D sa mg l tt | S lf th N | P l | S lf i N | P d | S lf m N m | P l | S lf d N m | P d | S lf th l N m | I d | S lfap d N m | I d | S lf l m t † N m e | P cure l | S lf so l ‡ N m | P cu d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2000 | — | — | — | — | — | — | — | — | — | — | 12 | 42 | 12 | 50 | 12 | 42 |
| 1000 | — | — | — | — | — | — | — | — | — | — | 12 | 33 | 12 | 25 | 12 | 33 |
| 500 | — | — | — | — | — | — | — | — | 24 | 58 | 12 | 0 | 12 | 0 | 12 | 0 |
| 250 | — | — | 12 | 67 | 12 | 83 | 42 | 48 | 24 | 46 | 12 | 0 | 12 | 0 | 12 | 0 |
| 125 | — | — | 12 | 67 | 12 | 83 | 18 | 67 | 24 | 33 | — | — | — | — | — | — |
| 63 | 12 | 83 | 24 | 58 | 12 | 67 | 18 | 22 | 24 | 21 | — | — | — | — | — | — |
| 31 | 12 | 83 | 12 | 50 | 12 | 17 | 18 | 0 | 24 | 4 | — | — | — | — | — | — |
| 16 | 24 | 17 | 12 | 0 | 12 | 0 | — | — | — | — | — | — | — | — | — | — |
| 8 | 12 | 0 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | MED = 2 95% C L 21 35 | | MED = 48 95% C L 29 79 | | MED = 56 95% C L 39 81 | | MED = 98 95% C L 57 164 | | MED = 300 95% C L 172 522 | | MED = 2000 C L not estimated | | MED = 2000 C L not estimated | | MED = 2000 C L not estimated | |

M th ts ed 4 d y d d d f t b l ted t l f l l t r d f t
† D t b d ed f Eyl d C l m 19 5
‡ M v b t d t m kg d y by m lt ply g by f t f 2

TABLE 2

TREATMENT OF EXPERIMENTAL TOXOPLASMOSIS IN THE RABBIT WITH SODIUM SULFADIAZINE

Rabbits were infected by the intracutaneous injection of 1000 *Toxoplasma* organisms Treatment was initiated either just after inoculation or at the time the fever reached 40 C (usually on day 6 of the infection)

| Dosage and day of treatment | No. rabbits | No. cured | Per cent cured | Days of death of dying animals |
|---|---|---|---|---|
| 500 mg per cent Na SD in water Rx—day 1 | 5 | 4 | 80 | 10 |
| 1000 mg per cent Na SD in water Rx—day 1 | 3 | 3 | 100 | — |
| 500 mg per cent Na SD in water Rx—1st day of fever | [illegible] | 4 | 57 | 10 10 10 |
| 1000 mg per cent Na SD in water Rx—1st day of fever | 3 | 1 | 33 | 10 18† |
| Untreated controls | 9 | 0 | 0 | 9 9 9 9 9 9 9 10 10 |

* T pl ma re i l t t ps th d ted by bold fa ed m l
† This animal probably did not d [illegible] toxoplasmosis

infected intracutaneously These experiments are summarized in TABLE 2 The drug was administered in the drinking water and administration was initiated either just after inoculation or at the first appearance of fever over 40 C (usually on the sixth day of the infection) The concentrations of drug used produced blood levels on the order of 4 to 8 mg per 100 ml of blood

When treatment was started just after inoculation most of the animals survived until sacrificed at about six weeks None of the mice inoculated with tissues died of toxoplasmosis The effect of treatment was to prevent or at least retard the development of a skin lesion as seen in untreated animals Fever was either prevented or of short duration

When treatment was delayed until fever was evident four of seven animals were cured *Toxoplasma* were not recovered when the survivors were sacrificed

All control animals died 9 to 10 days after inoculation showing typical skin lesions and other signs of toxoplasmosis

We have also reported treatment of a small number of Norway rats with sulfadiazine one week after infection [5] The rats were inoculated intraperitoneally and all control and treated rats survived At sacrifice *Toxoplasma* were recovered from the sulfadiazine treated rats as readily as from the controls thus indicating that the infection was not eradicated

*Action of the 2 4 diamino pyrimidines* Our laboratory in 1952 reported that 2 4-diamino 5 *p* chlorophenyl 6-ethyl pyrimidine or pyrimethamine known under the trade name of Daraprim exhibited marked activity against toxoplasmosis in mice in preliminary tests [29] This activity was independently discovered by Summers[30] and has been confirmed by several others [31-33] Activity was also found to be a characteristic of other related pyrimidines but pyrimethamine has so far proved to be the most active

In our experiments reported in greater detail in another paper [24] we found that gram for gram pyrimethamine was superior to sulfadiazine in curative

action against mouse toxoplasmosis Qualitatively the drugs were different in that pyrimethamine treated infections relapsed less frequently than sulfadiazine treated infections Also the time of relapse if it occurred, tended to be delayed in the sulfadiazine treated mice, but came soon after the termination of treatment in those given pyrimethamine

As with sulfadiazine we have found that toxoplasmosis can be cured with relative ease with an intraperitoneal inoculum of about 100 $LD_{50}$ units in mice whereas cure was not so readily achieved with an inoculum of 10 000 and 100 000 $LD_{50}$ Even so, significant numbers of mice were cured with the largest inoculum compared with a lesser number cured with sulfadiazine

Although superior on a gram for gram basis the curative dosage of pyrimethamine approaches the maximum tolerated level whereas sulfadiazine gives its maximum curative action at dosages much below toxic levels

*Synergistic action of pyrimethamine and sulfonamides* That pyrimethamine and sulfadiazine acted synergistically against toxoplasmosis in the mouse was reported in 1953 by our laboratory [34] and recently we reported much more detailed observations upon this subject [4] Since then this synergistic action has been confirmed by the laboratory of J K A Beverley in Sheffield England who very kindly made his results available to me prior to their publication [32]

In our experience the joint treatment has proved to be the best by far that we have achieved with any drugs or combinations With intraperitoneal inocula up to 100 000 $LD_{50}$ we have been able to cure more than half of the mice treated and with smaller inocula which probably more nearly represent the type which would be met naturally we have been able to cure nearly all mice treated The dosages at which cure was obtained are sufficiently low to indicate possible effective action in man For instance holding the sulfadiazine dosage at a level easily obtainable in man we obtained cure in most of the mice given a dosage of approximately 1 1 mg per kg per day This dosage would *correspond in man to a dosage of about 70 mg per day but it is quite* possible that levels similar to those in the mouse would be obtained at lesser dosages in the larger host

The degree of synergism between sulfadiazine and pyrimethamine is very great and it can be studied best perhaps by the methods developed by Finney [35] Comparison of the expected and observed potencies of the mixtures of the two drugs shown graphically in FIGURE 1 demonstrated that the mixtures had an effect more than six times as great as would have been expected from mere additive action Still following Finney a coefficient of synergism (K) of +10 2 was calculated This constant which is a measure of the degree of interaction of the drugs may become useful in the future in the comparative study of drug combinations

Although the interaction of pyrimethamine and sulfadiazine has been studied by us most thoroughly we have used pyrimethamine with both sulfapyrazine and sulfathiazole Synergism was easily demonstrable with these combinations but our data give some indications that the degree of interaction may differ between the combinations

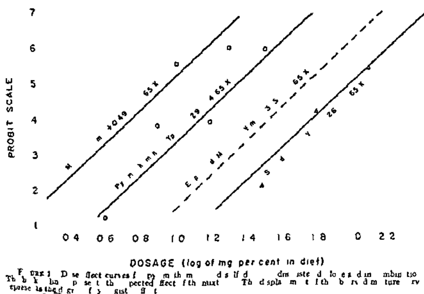
The outstanding activity of pyrimethamine and sulfadiazine against toxoplasmosis in mice led us to study this problem in other laboratory animals

These experiments are not complete as yet but some facts are becoming evident

In albino Norway rats inoculated intraperitoneally pyrimethamine and sulfadiazine eradicated the infection in most animals treated up to one week after inoculation [3] Since almost all rats survived the infection whether treated or not the effect was assessed by comparing the proportion of rats from which parasites could be recovered by subinoculation into susceptible mice Similarly, *Toxoplasma* could not be recovered from four treated puppies (less than six weeks old) whereas three of four untreated litter mates could be shown to be infected by subinoculation Because of the fact that persistent infections are readily induced in Norway rats this host has been used for a study of the effect of pyrimethamine and sulfadiazine on the chronic stages A large group of rats was treated after one or more months with dosages similar to those producing cure early in the infection At sacrifice organisms could be recovered from these rats as readily as from the untreated controls thus indicating no discernable effect for the drugs on this stage of the parasite [3]

Jacobs *et al* [36] have studied the production of acute toxoplasmic uveitis in rabbits and have used pyrimethamine and sulfadiazine in experiments on treatment Some benefit of treatment was demonstrable but the results were not consistently good Cook and Jacobs[37] also have studied the effect of pyrimethamine and sulfadiazine alone and in combination on *Toxoplasma* in roller tube tissue cultures Results parallel to those in animals were obtained and this technique of study should prove very useful in the future in the investigation of metabolite antimetabolite relationships as related to chemotherapy

Similar synergistic action of sulfonamides and pyrimidines has been observed in other *in vivo* systems Greenberg and Richeson[38] demonstrated synergism




F ure 1 D se ffect curves f py m th m d s lf d dm iste d lo e s d in mbin tio Th b k lin p se t th pected ffect f th mixt Th d spla m t f th b rs d m ture rv tpresse is the d gr f y gist ff t

between sulfadiazine and 2,4-diamino 5 aryloxypyrimidines in work on *Plasmodium gallinaceum* More recently, Lux[39] showed that sulfonamides and diaminopyrimidines and dihydrotriazines act synergistically against *Eimeria tenella* and suggested that economies in treatment might possibly be effected through the use of synergistic combinations

The explanation of the strong synergism between the sulfonamides and pyrimethamine is most likely that sequential blocks are produced along a single important metabolic pathway The role of the sulfonamides as antagonists of *p* aminobenzoic acid is well known and it is also well known that pyrimethamine has structural similarities to folic acid and sometimes acts as an antagonist of folic or of folinic acid

The work of Hitchings and his colleagues[40, 41] deals most thoroughly with the theoretical aspects of this problem and the *in vivo* studies such as ours on *Toxoplasma* are based materially upon their prior *in vitro* investigations The papers cited, as well as others by the same authors should be consulted by anyone with an interest in this field

*Other active drugs* A large number of drugs from groups other than the sulfonamides and the pyrimidines related to pyrimethamine have been tested in our laboratory in the laboratory of W A Summers and, to a lesser extent elsewhere Active compounds have been found in a number of groups but none of practical importance have been found except perhaps in the sulfone group

The sulfones were found to possess antitoxoplasmic activity by Biocca in 1943 and in a series of reports his group described activity for a number of compounds of this group including 4 4 diaminodiphenyl sulfone[27, 4, 46] Activity in this group has been confirmed by a number of investigators since the original report[3, 47, 48] The progress in studying this group up to 1952 was summarized by Eyles in a previous review[2] in which it was concluded that more work is necessary for proper evaluation

During the past three years we have performed extensive experiments using sulfones that a preliminary screening had shown to be particularly active against toxoplasmosis The data accumulated which are being prepared for publication indicated that none of the sulfones tested was superior in any way to the most active sulfonamides

As might be expected 4 4 diaminodiphenyl sulfone was among the most active and activity of the other sulfones for the most part was probably related to degradation in the host to this compound however a few compounds that could not by virtue of their structure thus degrade did show material activity

Beverley *et al*[28] have reported the use of 4 4 diaminodiphenyl sulfone in experimentally induced acute iritis in rabbits A beneficial effect was observed that was augmented by the use also of cortisone

That the mode of action of the sulfones is probably similar to that of the sulfonamides is demonstrated by the fact that synergism with pyrimethamine does occur Possibly some of the sulfones might be useful in cases in which known intolerance of the superior sulfonamides exists but the information we have

obtained so far leads us to disagree with Cross[2] who concluded that Diasone and Promin were more active than sulfadiazine

Antibiotics have been extensively tested against toxoplasmosis A number of these have been shown to be active to some degree the best probably being chlortetracycline (Aureomycin) The activity of this substance was discovered by Summers[47] and has been confirmed by several other investigators[17, 48-54] Varying degrees of activity have been described ranging from no effect (Adams[1] in embryonated eggs) to mere prolongation of life for a few days to eradication of the parasites

In our own work with this agent we have been able to produce apparent cures in more than half of a group of intraperitoneally infected mice given a diet containing 800 mg per cent of chlortetracycline for 14 days[54] All animals that survived 42 days appeared cured

On the other hand Steen[49] observed survival for 40 days in carrier state in mice given 0 1 mg *q i d* for 12 days Probably strain differences account for this and other discrepancies in the reports on this drug but certainly favorable effect is obtained only with enormous dosages much larger than those that could be attained in man

Chlortetracycline has been used unsuccessfully in at least two human cases of toxoplasmosis as will be pointed out later Mohr and Westphal[59, 60] reported the disappearance of dye test antibodies in man following the administration of chlortetracycline but Frenkel *et al*[61] and others were not able to confirm this finding

The other tetracyclines also exhibit activity Giroud and Gaillard[61] reported that oxytetracycline (Terramycin) at the rate of 100 mg per kg per day for three days prevented death in intradermally inoculated rabbits and was superior to chlortetracycline in this respect but Puech[6] reported negative results with somewhat smaller dosages On the other hand Grassi[5, 62] failed to find any activity for oxytetracycline in mice Our laboratory[63, 64] found that some prolongation of life was produced in mice by very large doses of this drug as did Garin[64] Cronroos[65] also observed weak activity in embryonated hens eggs Bogacz[1] found that oxytetracycline had activity in both rabbit and mouse infections Nobrega and Giovannoni,[66] using pigeons concluded that this drug produced a bacteriostaticlike effect that allowed development of immunity in treated animals Crema and Ambreck[67] observed little or no activity in mice

Tetracycline was found by our laboratory[54] to be definitely inferior to chlortetracycline on a gram for gram basis but did prolong the life of treated mice and this slight action was also observed by Palencia *et al*[67]

Cross and Joseph[68] reported slight prolongation of the life of mice treated with chloramphenicol (chloromycetin) and this was confirmed by our laboratory[63]

Christen and Thierman[69] found that puromycin known under the trade name Stylomycin and unfortunately for some time distributed under the name Achromycin produced prolongation of life in infected mice This observation was made independently by our laboratory[70] In unpublished studies our

laboratory has found that activity is possessed by a number of puromycin analogs and that the action is antagonized by large doses of adenine

Other antibiotics which have been found slightly active in mice include fumagillin[31, 53] and erythromycin[1, 72]. Bogacz[21, 73] reported that erythromycin and magnamycin were active against toxoplasmosis in rabbits and that spiromycin was active both in rabbit and mouse infections

Even though no antibiotic has yet become available with sufficient activity to be of practical importance in the treatment of toxoplasmosis slight activity has been found to be present widely in the antibiotic group. For this reason new compounds should be carefully tested as they become available

Another chemical group possessing antitoxoplasmic activity is the dihydro-s-triazines now being studied by Winters and Foley.[74] These compounds which have some antifolic acid properties also act synergistically with sulfonamides but insufficient information has been developed as yet to determine if practical importance will be possessed by this group

It is not within the scope of this review to list the large number of compounds that have been found to possess very slight antitoxoplasmic activity or the even larger number that have been found to possess none. For those interested in these compounds for theoretical reasons or as a guide for continued work on the chemotherapy of toxoplasmosis Summers and Eyles[75] have collaborated in the preparation of a summary of the screening work done in their laboratories and Eyles[2] presented some material on the minor drugs in a previous review

One generalization can be derived from the results on the variety of substances tried that is that antimalarial activity is very often associated with antitoxoplasmic activity although the reverse is not always the case. Illustrative of this general finding is the activity against both parasites of pyrimethamine sulfonamides sulfones compounds related to paludrine some quinolines and the naphthoquinone Lapinone. Also work so far indicates a parallel in activity against the two parasites among the antibiotics. Whether the above evidence of physiologic similarity is indicative of phylogenetic relationship remains to be determined

*The treatment of toxoplasmosis in man.* Evidence bearing upon the action of antitoxoplasmic drugs in man has accumulated slowly because of the scarcity of recognized cases and the difficulty of controlling the results of study. Despite these limitations some evidence as to the effect of drugs in man is becoming available

Sulfonamides have been used in a numer of cases of acquired toxoplasmosis with results that are difficult to evaluate. Kass *et al*[76] used sulfadiazine in one fatal case of acquired toxoplasmosis with no apparent benefit and this drug failed to check the course of a fatal infection reported by Sexton *et al*[77]. In this second case however medication was probably not started sufficiently early to give a fair trial. Sabin[78] used sulfanilamide during the final week in a case of encephalitis due to toxoplasmosis but the case terminated fatally although the fever abated somewhat for two days after initiation of the drug. Beverley *et al*[9] recently used sulfamethazine in a laboratory acquired case

that became fever free within five days but the authors stated no conclusion as to whether the effect of the therapy was warranted

In contrast Hormann[80] treated a laboratory infection with the sulfonamide preparation supracid (a mixture of sulfamerazine and globucid) and attributed the complication free recovery of his patient to this drug and earlier Franke and Horst[81] reported good results in several cases treated with another sulfonamide preparation solu supronal which also contains sulfamerazine Robinson[82] reported successful treatment with sulfathiazole and emetine in a peculiar case of meningoencephalitis with chorioretinitis In none of these cases was *Toxoplasma* recovered in laboratory animals although organisms were reported seen in all except that of Hormann in either spinal fluid smears or in preparations from skin lesions

Antibiotics have been used in a few cases of acquired toxoplasmosis Chlortetracycline failed in the case of Kass[8] and the case of Sexton[77] just as did sulfadiazine but Meira *et al*[83] reported favorable effect for chloramphenicol in a case in which typhoid fever was originally suspected More recently Michel *et al*[84] reported possible favorable results from the use of erythromycin in a case of adult toxoplasmosis but in these last two cases serologic evidence only was the basis of the diagnosis Biocca and Grieco[85] stated that they treated one case of human toxoplasmosis with complete success with 4 nitro-4-formyl aminodiphenyl sulfone but provided none of the particulars necessary for critical evaluation

The recognition of two laboratory cases of toxoplasmosis during the last two years has provided opportunity for testing the action of pyrimethamine and sulfonamides against acute adult toxoplasmosis One of these cases occurred in our laboratory[86] and I am indebted to Don Kayhoe Henry Beye and Leon Jacobs of the National Institutes of Health for information regarding the other[87]

In the case in our laboratory the worker became ill the principal symptoms being fever malaise and lymphadenopathy Parasites were isolated in mice from blood and from an excised lymph node Treatment was initiated within one week of the acute onset at about the time of the appearance of a rash An initial dose of 50 mg of pyrimethamine was followed by 25 mg in six hours and 25 mg per day thereafter for two weeks Along with the pyrimethamine 6 gm per day of a triple sulfonamide preparation containing equal parts of sulfadiazine sulfamerazine and sulfamethazine was also administered Fever disappeared within 24 to 36 hours and the other symptoms promptly abated The clinicians observing the patient were unanimously of the opinion that a therapeutic response had occurred

In the second case also proved by recovery in animals the course of events was very similar to that described above but the patient has not been observed sufficiently long to allow evaluation of the results of treatment

Although it is quite within the realm of possibility that spontaneous remission of symptoms occurred in our case the results are nonetheless encouraging and they constitute some evidence at least that these drugs are effective in acquired acute infections Petrovicky[88] in Czechoslovakia also recently re

ported the cure of a case of acute toxoplasmic meningoencephalitis in an adult with pyrimethamine and sulfonamides but this case was not parasitologically proved Petrovicky has informed me he has also been successful in other cases[89]

The results of several studies on the effect of pyrimethamine and sulfonamides against granulomatous uveitis and chorioretinitis have become available during the last year, and other studies are in progress The first report was that of Ryan and his associates[90] from the Clinical Center of the National Institutes of Health Twenty five of 29 cases presumed to represent toxoplasmosis showed improvement suggesting favorable response to the therapy Eight responded within one week and 17 within one month Since 3 of the 29 cases had been treated for only a short time only one case appeared to represent failure of the treatment Ryan s group provided data on toxic side effect and cautioned against the use of the drugs except under close medical supervision A number of dosage regimens were used including pyrimethamine at dosages up to 100 mg per day but no correlations between dosage and therapeutic effect were made

Cassady *et al*[91] in 1955 stated that they had treated 11 patients with dosages of 25 mg of pyrimethamine per 60 pounds body weight per day plus 1 gm of sulfadiazine per 60 pounds per day Results of the treatment were very good in most of the patients Cassady[9] however has informed me that lesions of some of his patients have shown renewed activity since the discontinuation of treatment In 1 of the 11 patients depression of leukocyte and platelet formation occurred with nearly disastrous results

More recently Hoover *et al*[93] reported on the treatment of 22 cases of presumed toxoplasmosis of the eye They observed some improvement in 13 including 4 in which excellent results were achieved No improvement was noted in six cases and three were doubtful These investigators employed 25 mg per day of pyrimethamine along with sulfonamides In spite of the lower dosage they had two cases of severe leukopenia requiring transfusion treatment

In Memphis our laboratory is cooperating with several ophthalmologists in studying cases presumed to be toxoplasmosis A number have been treated with pyrimethamine mostly at 50 mg per day plus triple sulfonamide or sulfadiazine at 4 gm per day Several patients have shown marked improvement which is believed to be due to the treatment No severe toxic side effects have been noted up to this time Details of one of these cases have been reported[94]

The experience so far with the treatment of toxoplasmosis of the eye would strongly suggest that success may be achieved at least in some cases Since the diagnosis is always presumptive and based in part at least upon the elimination of other possible causes it is very likely that misdiagnosed cases are frequently included in the treated groups Paradoxically success is apparently being obtained against the chronic ocular disease even though the drugs would not be expected to act against the pseudocyst stage of the parasite Probably this is an indication that the drugs act by eliminating foci of active multiplication For that reason it is not surprising that some recurrence of activity has

been observed in treated patients possibly as a result of renewed proliferation from remaining pseudocysts

Studies made so far of the treatment of toxoplasmic eye disease are subject to the valid criticism that formal control groups have not been maintained by the ophthalmologists parallel to the treated groups Thus the possibility that much if not all of the response may represent spontaneous remission remains All of the ophthalmologists involved in these studies however, have had experience with many untreated cases and are in a good position to evaluate the response observed Possibly this is a substitution of "art" for "science" and it is to be hoped that in the future controlled studies will be carried out

Before leaving the subject of the treatment of ocular toxoplasmosis attention should be called to the fact that pyrimethamine has been given in some of these cases at levels approaching those toxic to man In the more than 70 cases summarized there have been three cases of serious toxicity It should be emphasized that careful medical supervision is necessary and that frequent blood studies including platelet counts are essential if this drug is to be employed at high dosages More investigation is necessary to determine if lower dosages are effective as the work of Myatt *et al*[95] has indicated that 25 mg per day can be given for long periods without toxicity in most normal adults Preliminary experiments with mice indicate no interaction of pyrimethamine and the sulfonamides in the production of acute or chronic toxicity[24]

Our knowledge of the treatment of congenital toxoplasmosis is even more meager than that of the other manifestations of this disease Some attempts at therapy have been made particularly with sulfonamides but it is virtually impossible to evaluate the results In large measure this is due to the fact that by the time diagnosis is made in congenital cases there is usually extensive irreparable damage so that treatment could have only the result of preventing further damage It is to be hoped that earlier diagnosis may result in the recognition of some cases before extensive damage has taken place and that treatment may be attempted in these or that the disease may be recognized in the mother so as to allow treatment of the mother prior to birth

## *Summary and Conclusions*

Extensive experiments on the treatment of toxoplasmosis in laboratory animals are reviewed with the conclusion that there are two groups of drugs with promising activity These are the sulfonamides the most active of which are the sulfapyrimidines (sulfamethazine sulfamerazine and sulfadiazine) and sulfapyrazine The second group is the 2 4 diamino pyrimidines the most active of which is pyrimethamine *Less active compounds have been found* among the sulfones the antibiotics and other groups

Although possessing marked activity individually perhaps the outstanding characteristic of the sulfonamides and pyrimethamine is that they act together synergistically most likely by imposing sequential blocks upon the metabolic pathway involving para aminobenzoic acid folic acid and folinic acid This synergistic action makes it possible to obtain chemotherapeutic effect with much lower dosages than with the drugs individually

Parallel with the reports of outstanding activity in animals, reports are now appearing describing the use of sulfonamides and pyrimethamine in human toxoplasmosis Although the evaluation of these reports is very difficult it would appear that activity against both acute acquired toxoplasmosis and toxoplasmic uveitis and chorioretinitis is becoming reasonably well substantiated

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# ON THE NOMENCLATURE OF *BESNOITIA BESNOITI*, A PROTOZOAN PARASITE

*By* William L Jellison

*The United States Department of Health Education and Welfare Public Health Service National Institutes of Health National Microbiological Institute Rocky Mountain Laboratory Hamilton Mont*

The describing and naming of a new *Toxoplasma* like organism in the genus *Besnoitia* by Frenkel[16] (1953) has prompted an inquiry into the nomenclature of the genus and of *B besnoiti*, the only species previously known

The organism described by Frenkel, *Besnoitia jellisoni* came from a white footed mouse *Peromyscus maniculatus* in Lemhi County Idaho and has been successfully established and maintained in laboratory animals It has been the subject of two additional papers by Frenkel[16 17]

A review of the literature concerning the generic name *Besnoitia* and the species *B besnoiti* reveals an unusual amount of confusion that can be summarized and possibly clarified The exact author credit for the names still may be indefinite The pertinent literature on the subject is presented in chronological sequence with the writer's interpretation

1912 (November) A protozoan organism causing cutaneous and internal lesions in cattle in France (*une tache pyrénéene*) was studied and described by Besnoit and Robin[1] These investigators tentatively referred the organism to the genus *Sarcocystis* They realized that it probably was a new species They did not propose a name for it Other contributions on the subject by Besnoit and Robin and reviews of their paper are dated 1913[2 7] and 1914[8]

1912 Marotel discussed the original paper of Besnoit and Robin at some length and concluded his remarks with the following statements

Nothing similar has been found in animals

Messrs Besnoit and Robin therefore opened up a new field for this reason their names deserve to remain connected with the wonderful discovery and this is why I propose to designate their parasite with the name *Sarcocystis besnoiti* (Translated from the French )

This appears to be the first proposal of a species name and since it is accompanied by descriptive material the name is considered valid and has been accepted by later writers

1913 (March) At a meeting of the Société des Sciences Vétérinaires de Lyon held November 17 1912 the original paper by Besnoit and Robin was discussed along with other papers The published record of the meeting is signed by P Bru apparently secretary of the society Bru[4] records certain opinions of Marotel regarding the organism and quotes as well the two paragraphs I have given above In casual reading one might conclude that Bru is proposing the new name which is definitely not the case

1913 (May) Following the publication of Marotel s remarks by Bru dated March 1913, A Henry[5] commented on the nomenclature of the organism He discussed the characteristics of the organism used the genus name *Besnoitia* used the combination *Besnoitia besnoiti* and credited the authorship of the

species to Marotel In this contribution he stated In conclusion it would be prudent to consider, at least provisionally the protozoan of Messrs Besnoit and Robin as a particular type Our colleague Brumpt wishes to indicate to us that in a second edition of his *Précis de Parasitologie* in preparation he establishes the genus *Besnoitia* Brumpt for the parasite in question The latter must now be called *Besnoitia besnoiti* (Marotel 1913) (Translated from the French)

This short paper contains some description of the organism and gives characters to distinguish it from parasites in the genus *Sarcocystis* and *Globidium* Although Henry may not have intended to do so this would appear to establish and validate the generic name *Besnoitia* which would now be *Besnoitia* Henry 1913, regardless of later proposals

*1913* In the second edition of the *Précis de Parasitologie* referred to above by Henry Brumpt does not use or propose the generic name *Besnoitia* though he does refer to the organism On page 105 he refers to it as *Gastrocystis Robini* de la Vache (Besnoit and Robin)' He obviously errs in using the specific name *Robini* in place of *besnoiti* and he also errs in crediting the authorship to Besnoit and Robin It is entirely possible that Brumpt was merely citing the original paper by Besnoit and Robin without intending to credit Besnoit and Robin with authorship of the species name On page 113 Brumpt refers to the organism as *Gastrocystis besnoiti* (Marotel) and in doing so he employs the correct specific name Brumpt believes the change in generic assignment is justified

1916 Under the title Sur la sarcosporidiose bovine Franco and Borges present an extended and well illustrated contribution on this parasite They formally propose the genus name *Besnoitia* as they state It would be useful perhaps to create a new genus for which we propose the name *Besnoitia* which just as was noted by Henry was already suggested by Brumpt The parasite described by us in Portuguese cattle and that of the cutaneous sarcosporidiosis of Besnoit and Robin would now be *Besnoitia besnoiti* (Marotel 1912) (Translated from the French)

Franco and Borges were cognizant of the comments by Henry but apparently considered that his contribution did not validate the genus name Although this is the first formal proposal of the name *Besnoitia* the present writer is of the opinion that it is antedated by *Besnoitia* Henry 1913 and therefore unnecessary It is fortunate that they agreed on the same genus name

1916 The Zoological Record contains the entry *Besnoitia* gen n for *Sarcocystis besnoii* from cattle Franco and Borges Arq Inst Bact Camara Pestana 4 p 269 [9] There is no previous entry to *Sarcocystis besnoiti* in the Zoological Record (1912–1916) [10] so there is no indication here as to whom the species is to be credited

1926 Wenyon[11] changes the name of the organism to *Globidium besnoiti* (Marotel) and states It is evidently very similar to the members of the genus *Globidium* in which it seems better to retain it at present as *G besnoiti*

1927 Brumpt[12] follows Wenyon in the change of the generic assignment but errs again in crediting the authorship to Besnoit and Robin instead of to Marotel for he lists *Globidium besnoiti* de la Vache (Besnoit et Robin)

1939 The Nomenclator Zoologicus by Neave[13] contains the entry '*Besnoitia* Franco and Borges 1916 ——— '

1949 Brumpt[14] in a sixth edition of his *Précis* used the combination *Besnoitia besnoiti* ' for the legend of an illustration on page 62 He also refers to *Globidium besnoiti* in his index referring to page 536, but the species is not mentioned on that page On page 537 he lists *Globidium besnoiti* de la Vache (Besnoit et Robin) ' so he is at least consistent in the error of crediting Besnoit and Robin with the authorship of the species name

1953 Frenkel[15] employs the generic name *Besnoitia* in describing a new species *B jellisoni* from mice

On the basis of this chronology the name *Sarcocystis besnoiti* dates to Marotel 1912 The generic name *Besnoitia*, dates to Henry 1913 and the correct combination is *Besnoitia besnoiti* (Marotel, 1912) Henry, 1913

The present writer offers no opinion on whether or not these two species deserve the distinction of a genus separate from *Gastrocystis Globidium*, or *Sarcocystis*

*Acknowledgments*

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# TRANSMISSION OF THE PROTOZOAN *BESNOITIA JELLISONI* BY INGESTION

*By* William L Jellison W J Fullerton, and Hazel Parker

*The United States Department of Health Education and Welfare Public Health Service National Institutes of Health National Microbiological Institute Rocky Mountain Laboratory Hamilton Mont*

A *Toxoplasma* like organism infecting a wild mouse *Peromyscus maniculatus* in Lemhi County, Idaho was described and named *Besnoitia jellisoni* by Frenkel (1953) Frenkel (1953 1955) was able to establish and maintain this parasite in laboratory white mice in which it produced acute fatal disease in some, and chronic infections with development of typical globidial cysts in others

Interest in this organism is due to its similarity in certain stages to *Toxoplasma gondii* and to its relationship to *Besnoitia besnoiti* (Marotel) which is an important parasite of domestic cattle

The mode of infection in nature with *B besnoiti* is unknown It has recently been transmitted experimentally to cattle and also to rabbits (Pols 1954a 1954b) Pols (1954a) states The maintenance of *Gl besnoiti* [= *B besnoiti*] in rabbits will greatly facilitate studies on the life cycle of this parasite determine the host range and evaluation of chemotherapeutic agents

The discovery of a new member of this genus as a natural parasite of wild mice and as one readily adaptable to laboratory mice provides another tool for a study of the life history and pathogenicity of this important group of pathogens which includes *Globidium Sarcocystis Toxoplasma* and *Besnoitia*

*Besnoitia jellisoni* is easily transmitted to mice and to a wide variety of other rodents and rabbits by intraperitoneal or intravenous inoculation of peritoneal fluid from an acutely ill animal or with organisms from cysts in an animal with chronic infection These modes of transmission however would not account for maintenance in nature Preliminary observations in the laboratory suggested that transmission sometimes occurs through ingestion and as wild mice are known to be cannibalistic this would account for some transmission in nature To determine the effectiveness of transmission by ingestion two experiments were performed In one experiment the peritoneal fluid from a moribund white mouse was fed to litters of white mice and in the other organisms from cysts in an experimentally infected *Peromyscus* were fed to litters of white mice The details and results of these experiments follow

The baby mice were held under a binocular microscope for better observation while the suspension was administered orally with a capillary pipette Care was taken not to traumatize the mouth area Only a drop or two of suspension was taken by the smaller mice When the young mice were replaced with the female she licked and cleaned them immediately and in this way was exposed to infection Also as the young became sick a few were eaten by the females

*Experiment I Transmission by feeding organisms from cysts* On March 10 1955 an adult white footed mouse *Peromyscus maniculatus* inoculated over five months previously with *B jellisoni* by intraperitoneal injection was sacrificed It contained numerous cysts filled with the slender crescent shaped or characteristic of the globidial stage of the infection Cysts were

TABLE 1

RESULTS OF FEEDING BESNOITIA ORGANISMS FROM CYSTS TO MICE

| Litter age in days | Maternal ♀ | Young mice A | B | C | D | E | F | Proportion infected |
|---|---|---|---|---|---|---|---|---|
| 1 | S 46+ | M | M | M | M | D 19+ | S 19+ | ♀ and 2/6 |
| 3 | D 14+ | D 8− | D 13+ | D 13+ | D 14− | M | S 46− | ♀ and 2/6 |
| 5 | S 46+ | D 11+ | D 13− | D 13+ | D 13+ | D 13− | D 17+ | ♀ and 4/6 |
| 7 | D 11+ | D 15+ | D 15+ | D 16− | D 18− | M | S 46+ | ♀ and 3/6 |
| 9 | D 21− | D 15+ | S 33+ | D 45+ | S 46+ | S 46+ | S 46+ | 6/6 |
| 11 or 12 | S 46+ | M | S 33− | S 46+ | S 46+ | S 46+ | S 46+ | ♀ and 4/6 |
| 13 | S 47− | M | S 33− | S 47+ | S 47− | S 47+ | S 47− | 2/6 |
| 15 | D 15+ | D 24+ | D 24+ | D 24− | D 29− | D 30− | D 36+ | ♀ and 3/6 |
| 17 | D 18+ | D 14+ | D 26+ | D 29− | S 34+ | S 47+ | S 47+ | ♀ and 5/6 |
| 19 | S 47+ | D 16+ | D 21+ | S 47+ | S 47+ | S 47+ | S 47+ | ♀ and 6/6 |

Legend S = survived D = died − = negative for organisms or cysts + = positive for organisms or cysts M = missing and number = days survived prior to autopsy

present in the subcutaneous tissues peritoneum lungs, and pleural cavity A feeding experiment was performed using 10 litters of white mice ranging in age from 1 to 19 days Each litter contained 6 young Individual cysts were removed from the infected mouse and their contents were teased out into a drop of chick embryo juice Within one minute after the cyst was ruptured the drop was picked up with a capillary pipette and delivered into the mouth of a baby mouse Each baby mouse received the contents of one cyst Ten litters of white mice ranging in age from 1 to 19 days were fed in this manner Each litter contained 6 mice indicated as A B, C D, E and F in TABLE 1

The results of this experiment are summarized in TABLE 1 In no instance was the maternal female in the litters given the infective suspension intentionally but she experienced exposure in grooming the young and in some instances in eating the young that died Five of the 10 females died during the course of the experiment Organisms were present in either or both, the pleural or peritoneal fluids of four One of the five females that died was decomposed and organisms were not found Five females survived the course of the experiment Of these four were infected and they exhibited numerous globidial cysts when sacrificed on the 46th or 47th day of the experiment One of the surviving females showed no sign of infection when sacrificed on the 47th day Thirty seven of the 60 young experimental mice became infected by ingestion of a suspension of organisms teased from cysts Mice of all ages from 1 to 19 days became infected At least two out of each litter of six were positive at death or when sacrificed at the conclusion of the experiment In the 9 and 19-day-old litters every mouse became infected Mice in this experiment that were infected and survived to the 29th day showed the cystic or globidial stage of the organism when autopsied

*Experiment 2 Transmission by feeding organisms from peritoneal fluid* On March 10 19[illegible] a transfer of the organism was made from an infected mouse to four mature white mice by intraperitoneal inoculation Seven days later

TABLE 2

RESULTS OF FEEDING PERITONEAL FLUID FROM A BESNOITIA INFECTED MICE TO YOUNG MICE

| Litter age in days | Maternal ♀ | Young mice | | | | | | | Infected |
|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | |
| 1 | S 46− | M | M | M | M | M | S 46+ | S 46+ | ?/ |
| 3 | S 46− | D 14+ | D 33− | D 33+ | D 34+ | D 34− | M | S 46− | 3/ |
| 5 | S 46− | M | M | M | S 46+ | S 46+ | S 46+ | S 46+ | 4/7 |
| 7 | S 46− | D 36− | D 38− | M | M | S 46− | S 46− | | 0/6 |
| 9 | S 46− | M | M | M | S 3− | S 46+ | S 46+ | S 46+ | 3/7 |
| 11 | D 22− | S 46− | S 46− | S 46− | S 46− | S 46− | S 46+ | S 46+ | 2/? |
| 13 | S 46− | S 46− | S 46− | S 46− | S 46− | S 46− | S 46− | S 46− | 0 |
| 15 | S 46− | S 46− | S 46− | S 46− | S 46− | S 46− | S 46+ | S 46+ | 2/7 |
| 16 | M | S 46+ | S 46− | S 46− | S 46− | S 46− | | | 1 ? |
| 20 | S 46− | S 46− | S 46− | S 46− | S 46− | S 46− | S 46− | | 0/6 |

Legend S = survived D = died − = negative for organisms or cysts + = positive for organisms or cysts M = missing and numeral = day survived to autopsy

one mouse was dead and the others were sick. One of the sick mice was sacrificed and about 4 cc of peritoneal fluid were harvested. This fluid was rich in organisms, and two separate counts using a hemocytometer gave 1,500 and 2,350 organisms per cmm respectively. The peritoneal fluid was used without dilution or concentration to feed 10 litters (5 to 7 young per litter) of white mice ranging in age from 1 to 20 days.

The results of this experiment are summarized in TABLE 2. Again the mother mice were not intentionally infected but experienced exposure in grooming their young or eating young that died. One female died during the course of the experiment but infection was not demonstrated. A second was missing. The remaining eight were sacrificed at the conclusion of the experiment on the 46th day and had no evidence of infection. By this time infection, if present, would have been manifested by the presence of cysts.

In two litters 7 days and 20 days of age no infection resulted. One or more mice in each of the other litters became infected. The greatest proportion of infection observed was in the 5-day-old litter where 4 of 7 became infected. Seventeen of 66 of the young mice became infected as evidenced by finding organisms in the pleural or peritoneal fluid at time of death or by finding cysts in the tissues after the 33rd day of the experiment or at its conclusion on the 46th day. This infection rate is about one half that obtained in experiment 1 where organisms from cysts were used for inoculum. The greatest contrast is that none of the maternal females in experiment 2 were known to have become infected, whereas 8 of 10 females in experiment 1 became infected.

This difference, which is not especially significant in the case of the young, may be attributed to the stage of the organism used for inoculum, the concentration of organisms in the inoculum or other factors.

The results are adequate proof that *B jellisoni* can be transmitted to mice by ingestion and this method could be effective in nature as mice are known to be cannibalistic.

TABLE 1

RESULTS OF FEEDING BESNOITIA ORGANISMS FROM CYSTS TO MICE

| Litter age in days | Maternal ♀ | Young mice | | | | | | Proport infected |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | |
| 1 | S 46+ | M | M | M | M | D 19+ | S 19+ | ♀ and 2/6 |
| 3 | D 14+ | D 8− | D 13+ | D 13+ | D 14− | M | S 46− | ♀ and 2/6 |
| 5 | S 46+ | D 11+ | D 13− | D 13+ | D 13+ | D 13− | D 17+ | ♀ and 4/6 |
| 7 | D 11+ | D 15+ | D 15+ | D 16− | D 18− | M | S 46+ | ♀ and 3/6 |
| 9 | D 21− | D 15+ | S 33+ | D 45+ | S 46+ | S 46+ | S 46+ | 6/6 |
| 11 or 12 | S 46+ | M | S 33− | S 46+ | S 46+ | S 46+ | S 46+ | ♀ and 4/6 |
| 13 | S 47− | M | S 33− | S 47+ | S 47− | S 47+ | S 47− | 2/6 |
| 15 | D 15+ | D 24+ | D 24+ | D 24− | D 29− | D 30− | D 36+ | ♀ and 3/6 |
| 17 | D 18+ | D 14+ | D 26+ | D 29− | S 34+ | S 47+ | S 47+ | ♀ and 5/6 |
| 19 | S 47+ | D 16+ | D 21+ | S 47+ | S 47+ | S 47+ | S 47+ | ♀ and 6/6 |

Legend S = survived D = died − = negative for organisms or cysts + = positive for organisms or cysts M = missing and numeral = days survived to autopsy

present in the subcutaneous tissues peritoneum lungs and pleural cavity A feeding experiment was performed using 10 litters of white mice ranging in age from 1 to 19 days Each litter contained 6 young Individual cysts were removed from the infected mouse and their contents were teased out into a drop of chick embryo juice Within one minute after the cyst was ruptured the drop was picked up with a capillary pipette and delivered into the mouth of a baby mouse Each baby mouse received the contents of one cyst Ten litters of white mice ranging in age from 1 to 19 days were fed in this manner Each litter contained 6 mice indicated as A B C D E and F in TABLE 1

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*Experiment 2 Transmission by feeding organisms from peritoneal fluid* On March 10 1955 a transfer of the organism was made from an infected mouse to four mature white mice by intraperitoneal inoculation Seven days later

# IOXOPLASMOSIS SUMMARY AND CHALLENGE

*By* Don L I yles

*The U ited States Department of Health Educati n and Welfare Public Health Service National Institute of Allergy and Infecti us Diseases Nati al Micr bi l gical Instit te Lab ratory of Tropical Diseases at the Medical School of the Uni ersity f Tennessee Memphis Tenn*

Although the parasite *Toxoplasma gondii* has been recognized for 41 years or more it is only since Wolf and his colleagues discovered its association with human disease that extensive research on it has been conducted Thus we are dealing with a subject that is essentially just 16 years old and one in which progress can be seen easily from year to year The tremendous recent activity in research on toxoplasmosis was demonstrated well by Markham s analysis in the introduction to this symposium

The preparation of this summary is particularly interesting to me for the reason that just three years ago this month, I participated along with others contributing to this publication in a conference similar to that upon which this monograph is based During the period since the previous review much progress along certain lines has been made but along others much remains to be done A great mass of information has been presented to you thus far in these pages so in my summary I shall attempt to touch merely on the high points and I shall emphasize the challenge that remains before us

Among the outstanding recent accomplishments to which we may point in this summary is the progress that has been made toward defining the role of toxoplasmosis as a cause of disease manifestations with which it had previously not been associated Specifically I refer to the elucidation of the role of toxoplasmosis in granulomatous uveitis alluded to by Jacobs and to the work of Siim upon acquired toxoplasmosis

The solution of the puzzle posed by adult granulomatous uveitis is an example of the best in cooperative activity between parasitologist clinician and pathologist The discovery of forms resembling *Toxoplasma* by the alert Miss Wilder followed by the thorough and convincing serologic and parasitologic studies of Jacobs along with careful analyses by several ophthalmologists has persuaded most of us that *Toxoplasma* is a very important cause of acquired uveitis and chorioretinitis and that this form of the disease perhaps constitutes its most important human manifestation

Similarly the brilliant work of Siim and his colleagues has made it clear that acquired lymphadenitis associated with toxoplasmosis is a real entity and it remains only for us to determine with what relative frequency this form of the disease prevails We must find out if the small number of such cases seen so far in the United States is due to failure to recognize these cases or if it is due to a difference in the frequency of occurrence Particularly interesting is the case cited by Siim that of a child with toxoplasmosis born to a mother believed to have had a toxoplasmic lymphadenopathy during pregnancy

This case and that of Alexander mentioned by Frenkel are the first cases known to me of association of clinical toxoplasmosis in the mother with the birth of children with toxoplasmosis

## *References*


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Finally, fundamental work on the mode of action of active compounds should be continued as the information obtained may be useful in pointing the way toward other practically effective agents and will certainly provide insight into basic physiologic processes applicable not only to *Toxoplasma* but, perhaps to other organisms as well

Although the information presented by several contributors to this monograph has had bearing upon the epidemiology of toxoplasmosis it is significant that no paper solely on this subject has been proffered This is a reflection of the lack of progress toward determining the mode of transmission of toxoplasmosis and it is in this area that the outstanding challenge to us remains Certain hypotheses have been advanced with some supporting evidence, but none is in accord with all of the facts known about the parasite and its pattern of occurrence in its variety of hosts Some investigators postulate multiple modes of transmission, and this assumption may well be a fact but we must guard against assuming this solely through the necessity of explaining observations not in accord with our favored hypothesis

Continued studies such as those of Feldman and others on the occurrence of serologic reactors in different population groups may ultimately provide leads as to possible modes of transmission Other studies of the association of man with possible host animals are also in order and these investigations may provide pertinent information In the meantime a fertile field for investigators with imagination continues to exist

I find that in this summary and challenge I have emphasized the challenge at the expense of the summary I have done this because I believe that one of the most important results of a publication such as this is to point the way toward further progress The points of view presented and the discussion recorded in these pages should enable us all to reorient our thinking and above all should stimulate us to renewed activity in solving the many remaining problems

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The notable progress exemplified by the work of Jacobs and his associates and of Sim should stimulate us to investigate vigorously the extent to which these forms of the disease occur, and to study the possible relationship of toxoplasmosis to other disease entities

Feldman has brought us up to date on the present knowledge of congenital toxoplasmosis Extensive research by him and by others has brought us a clear understanding of this disease entity We know that, although probably infrequent this manifestation of the disease is nearly always accompanied by serious residual damage but even more important we have learned from this work that mothers of toxoplasmic children can bear subsequent children with confidence that no tragic results will be produced by toxoplasmosis

In the future perhaps we can hope to discover some means of detecting the disease in the mothers and a method of treatment that will prevent the development of toxoplasmosis in the child

Therefore laboratory diagnosis discussed by Eichenwald constitutes one of our major problems Progress to date has been material For instance the development of the dye test by Sabin and Feldman was a milestone in the history of toxoplasmosis The use of this test has been of greatest importance in dealing with this disease in which it is so difficult to isolate the actual etiologic agent

Even so I believe that the refinement of the dye test and the devising of other diagnostic methods should be a major field of research on this disease We need more information on the specificity of the dye test and of the skin test as is evident from the discussions recorded in these pages Perhaps Eichenwald s results with tissue culture techniques may provide a useful tool for research and for diagnosis and we can hope also for the development of more simple efficient and economic quantitative methods perhaps using dead antigens that would eliminate the cumbersome and dangerous necessity for maintaining strains of *Toxoplasma* in diagnostic laboratories

Frenkel has outlined for us the present knowledge of the pathogenesis of toxoplasmosis and has pointed out the problems to be pursued among which are the differentiation of lesions produced by cyst rupture or by proliferation of organisms Also reported by Frenkel was recent work on organisms related to *Toxoplasma* or organisms with which it may be confused Certainly further work on these forms is needed

In the treatment of toxoplasmosis there have been material accomplishments particularly in the chemotherapy of experimental toxoplasmosis Even so further effort is necessary along several lines

First better evaluation of the practical usefulness in man of the drugs we now have is an essential line of endeavor and one that presents a most challenging problem Previously I have alluded to the great difficulty of evaluating the observed effects In spite of the difficulty of establishing satisfactory controls the effectiveness of pyrimethamine and sulfonamides or combinations must be assessed in acquired infection and ocular toxoplasmosis I have mentioned the dearth of data on the treatment of congenital toxoplasmosis

Second search should be continued for better agents particularly for agents that may have an effect upon the pseudocyst stage